THERMOREGULATION IN THE SOUTH AMERICAN GREY SHORT-TAILED OPOSSUM, *Monodelphis domestica*:

THE EFFECTS OF AMBIENT TEMPERATURE, BACTERIAL ENDOTOXIN AND HYPOXIA ON BEHAVIOURAL AND AUTONOMIC BODY TEMPERATURE CONTROL

Volume I: Thesis

By

Tracy Douglas (B.Sc. Hons)

* * * *

A thesis submitted in fulfilment of the requirements for the degree of Master of Medical Science (M.Med.Sc)

University of Tasmania

August, 2003

DECLARATION

I declare that this thesis does not include any material which has been accepted for a degree or diploma by this university or any other tertiary institution except by way of background information which is duly acknowledged in the thesis. In addition, to the best of my knowledge and belief, this thesis does not contain any material previously written or published by another person except where due reference is made. Finally, I certify that I performed all reported experiments in this thesis.

Tracy Douglas B.Sc. (hons)

STATEMENT OF AUTHORITY OF ACCESS

This thesis may be made available for loan and limited copying in accordance with the *Copyright Act 1968.*

L A Douglas.

25/8/03

ABSTRACT

This study investigated the concept of thermoregulatory set-point in marsupials, by examining behavioural and autonomic thermoregulation in a laboratory-bred marsupial, the South American grey short-tailed opossum (*Monodelphis domestica*) with occasional reference to a single captive sugar glider (*Petaurus breviceps*).

Adult animals had clear circadian rhythms of core body temperature (Tb). Such rhythms were not apparent in juvenile *M. domestica* and rhythms in adult animals were disrupted when ambient temperature (Ta) was reduced, bacterial endotoxin (*E. coli* LPS) was injected and ambient oxygen levels were lowered to approximately 10% (i.e. during hypoxia).

Circadian changes in preferred Ta were observed in individual animals while in a longitudinal thermal gradient but no specific rhythm was observed. *M. domestica* was able to make small, but insignificant, changes to Tb using thermoregulatory behaviour. This resulted in less variance in Tb in this species while in a thermal gradient.

A typical mammalian fever to LPS and a typical hypothermic response to hypoxia was observed in *M. domestica* and one *P. breviceps*. Although hypothermic responses to hypoxia have been previously documented in marsupials, this is the first published account of LPS-induced fever in marsupials. Preferred Ta was not significantly affected by hypoxia or fever in *M. domestica* although a significant reduction in preferred Ta was observed in the sugar glider while hypoxic. This animal also selected a warmer environment while febrile. The hypothermic response to hypoxia in *M. domestica* was found to be more significant than the hyperthermic response to bacterial endotoxin. However, the sugar glider utilised thermoregulatory behaviour to maintain a hyperthermic response to bacterial endotoxin even when exposed to hypoxia.

These findings show that in marsupials, as in eutherians, thermoregulation involves the interaction of autonomic and behavioural mechanisms. These experiments also highlighted the limitations of using laboratory bred animals to determine the natural thermoregulatory capacities of a species.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Assoc. Prof. Stewart Nicol for his expert surgical skills, guidance and support throughout the years of this study. His valuable assistance and critical review of this thesis have also been greatly appreciated. Special thanks also to David Lovell for his eager assistance during surgical procedures and to Dr Niels Andersen for his willing assistance throughout many aspects of the experimental work.

I am particularly grateful to the animal house staff at the University of Tasmania for their assistance and husbandry of animals. In particular, I would like to express particular thanks to David Jacobs and Karen Lomasney for their assistance with the South American opossums and Eileen Wronski who offered valuable assistance with the sugar gliders and their requirements. In addition, Ron Mawbey from the Department of Zoology provided invaluable guidance with respect to the capture and release of the sugar gliders. Capture sites were kindly offered by Stuart and Gill Auckland and Peter Dixon on their private properties.

Experimental assistance with the opossums was given by Sharon Monk and Janne Bailey assisted with experimental procedures with the sugar gliders. The behavioural thermoregulation studies were made possible due to the generous loan of the thermal gradient by Dr Peter Frappell, LaTrobe University.

I am particularly grateful to my work colleagues at the School of Human Life Sciences for their support and friendship throughout this study. I would particularly like to acknowledge the contributions made by Mr Bryan Day, Dr Phyllis Mooney and Dr Dominic Geraghty with respect to the provisions of experimental equipment, assistance with the sugar glider and their encouragement throughout the time of this study.

Any in-depth study cannot be achieved without the sincere support of family and close friends. My parents have shown keen interest and support throughout my academic career and I thank them for this wholeheartedly. My husband, Andrew has been a pillar of support and has made many aspects of this work successful. His patience, encouragement, and willing assistance with the trapping of sugar gliders have helped make this study possible.

Finally, these acknowledgements would not be complete without mentioning the animals which made this study both possible and enjoyable. The eighteen opossums, in particular, "Lenny" and "Pedro", were a delight to handle and work with experimentally and the sugar glider which was captured and re-released during the study provided some particularly memorable moments.

This study was made possible due to the approval of the Animal Ethics Committee of the University of Tasmania and the National Parks and Wildlife Service of Tasmania.

LIST OF ABBREVIATIONS

BAT	brown adipose tissue
CO_2	carbon dioxide
i.m.	intramuscular
LPS	lipopolysaccharide
MR	metabolic rate
NST	non-shivering thermogenesis
O_2	oxygen
PG	prostaglandin
Selected Ta	selected ambient temperature
T_3	triiodothyronine
T_4	thyroxine
Ta	ambient temperature
Tb	core body temperature
TNZ	thermoneutral zone
ΔTa	difference in ambient temperature
ΔTb	difference in body temperature

DEDICATION

THIS THESIS IS DEDICATED TO

TEGAN

WHOSE LOVE OF ANIMALS
WILL NEVER BE FORGOTTEN

and

My husband, Andrew
in appreciation of his love and support throughout the research and preparation of this thesis

TABLE OF CONTENTS

Volume I: Thesis

Page

PREFACE

The following study was designed to investigate aspects of autonomic and behavioural thermoregulation in two small, non-macropod marsupial species; one, a species widely kept as a laboratory animal, the other, a wild species.

The first series of experiments involved the South American short-tailed opossum, *Monodelphis domestica*. These animals were obtained from a laboratory colony; bred and maintained in a constant temperature room (28-30°C). A total of eighteen animals were used. Circadian rhythms of body temperature were measured in individual animals under normal conditions (i.e. constant temperature room) using telemetric techniques. The ability of animals from this colony to respond to cold was then investigated and levels of thyroid hormones measured. Using a longitudinal thermal gradient, behavioural thermoregulation was then studied in these laboratory animals and the effects of bacterial endotoxin and hypoxia on rhythms of core body temperature and selected ambient temperature determined.

The second series of experiments involved a marsupial captured from its natural habitat, the sugar glider, *Petaurus breviceps*. This is an Australian marsupial of similar size to *Monodelphis domestica*. Using telemetry, circadian rhythms of core body temperature were measured in this species and behavioural thermoregulation was analysed using a longitudinal thermal gradient. The effects of bacterial endotoxin and hypoxia on rhythms of core body temperature and selected ambient temperature in *Petaurus breviceps* were then investigated.

Traps were set to capture *Petaurus breviceps* over a total period of four months with up to four traps set simultaneously and traps were monitored daily (a total of 112 nights of active trapping). In addition, traps were baited but not set for a total period of nine months to attract sugar gliders to the traps

and determine whether any animals existed within the trapping area (during this period one brushtail possum became caught in a trap but was successfully released and traps were knocked out of trees, supposedly by possums, on a couple of occasions). A number of trapping sites were used in areas where sightings of the species had been made. Unfortunately, despite exhaustive efforts, only one female animal was successfully trapped and consequently used in the designed experimental protocol.

It was hoped that this study would determine any differences in thermoregulation in a laboratory marsupial species compared to a wild marsupial species. Unfortunately, the problems encountered in trying to obtain the wild species limited the scope of this study yet still highlighted interesting points concerning the legitimacy of thermoregulatory data obtained purely from animals which have been bred and maintained in an artificial environment. The data have been presented and discussed as a thermoregulatory investigation of *Monodelphis domestica*. Occasional reference has been made to data from the single *Petaurus breviceps* throughout the thesis.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Body temperature (Tb) regulation is an important tool for animals exposed to fluctuating ambient conditions. Claude Bernard was the first to formally describe Tb regulation as a homeostatic process that maintains the stability of the internal environment (as cited by Jansky, 1995). Since Bernard's formal description, the concept of a biological set-point of Tb has been proposed, homeostatically controlled by both voluntary and involuntary processes (see Hammel, 1970).

Circadian variations of core Tb have been observed in many animals with a daily peak or maximum in Tb detectable in diurnal and nocturnal species (eg Fuller and Sulzman, 1982; Refinetti and Menaker, 1992b; Refinetti, 1996). This increase has been shown to be associated with autonomic (physiological) and behavioural (voluntary) responses which facilitate heat production and conservation. Thus, a relatively constant internal Tb is continuously maintained.

Mammals and birds share a common ability to regulate core Tb irrespective of environmental temperatures (Herrington, 1940) and are thus referred to as endotherms or homeotherms. The evolution of endothermy is believed to have involved either an increase in aerobic capacity (eg Bennett, 1991) or an enhanced thermoregulatory capacity involving the expansion of ecological niches (eg Crompton et al., 1978). Ultimately, higher Tbs and metabolic rates were then attained by both birds and mammals (see Ruben, 1996). Generally, birds have higher Tbs than mammals during both active and resting phases (Prinzinger et al., 1991). In addition, marsupial (pouched) and monotreme (egg-laying) mammals have lower levels of energy metabolism than eutherian (placental) mammals (eg Dawson and

Hulbert, 1970). Lovegrove (2000) suggests that the differences in metabolic rate are based on ecological zone rather than phylogeny.

Many lower vertebrates (ie reptiles, amphibians and fish) are regarded to be ectothermic yet may regulate core Tb by behavioural as well as physiological means (eg, Sievert and Hutchison, 1988). When placed in an experimental temperature gradient ectotherms will move to an ambient temperature (Ta) that enables them to attain a selected Tb. Mammals and birds also utilise behavioural as well as physiological means to regulate Tb. This combination of behavioural and physiological regulation of Tb in endotherms is precise and within narrow limits.

Deep Tb is governed by a complex combination of physical (ie Ta, photoperiod and altitude) and chemical factors (ie electrolytes, neurotransmitters and synthetic drugs). According to Gordon (1983a) Tb can change if there is an alteration in the "set-point" temperature and/or an excessive endogenous or environmental heat load overwhelms the capacity to thermoregulate. This is termed regulated and/or forced shifts in Tb. Fever is an example of a regulated change in Tb (Gordon, 1983a). "Set-point" refers to a regulated or selected Tb level.

Hammel et al., (1963) first postulated an adjustable thermal set-point in the dog. In endotherms, it is proposed that the set-point is determined by the hypothalamus which contains the thermal control centres (Bligh, 1966). Core Tb is regulated through various mechanisms of heat gain and heat loss such as shivering and evaporation (Hammel, 1968). As a result, the set-point temperature is homeostatically maintained (Hammel, 1970).

1.2 Set-Point Theory of Thermoregulation

Circadian variations in endothermic core Tb exist and are proposed to be due to cyclic shifts in the "set-point" of the endothermic thermoregulatory system (eg Hensel, 1973). The set-point theory of thermoregulation

proposes that there is an in-built or reference value for Tb which forms the basis of thermal homeostasis (Hammel et al., 1963). This set-point is believed to be located in the hypothalamic region of the mammalian brain (Hensel, 1973; Osborne and Refinetti, 1995). The pre-optic region of the hypothalamus acts as a thermostat in amphibians and reptiles (Berk and Heath, 1975) thus on an evolutionary basis it is highly likely that the set-point theory applies to marsupials and monotremes as well as eutherian mammals (Baudinette, 1984).

A shift in set-point temperature will occur if body core temperature is changed and maintained by appropriate thermogenic responses (see Satinoff and Henderson, 1977). For example, a shift to a higher set-point temperature encourages an organism to produce and retain more heat and therefore behaviourally seek a warmer environment. A shift to a lower set-point has a converse effect physiologically and behaviourally. The set-point theory of thermoregulation is a descriptive concept and can be readily investigated by comparing changes in core Tb with autonomic and behavioural responses (Hensel, 1981). Such investigations have been carried out in birds (eg Nichelmann et al., 1999) and eutherian mammals such as rats (eg Gordon, 1994), but are yet to be discussed in marsupial or monotreme mammals.

According to the adjustable set-point model, the thermoregulatory response is proportional to the difference between the actual hypothalamic temperature and the set-point temperature at which internal Tb is regulated (Corbit, 1970). Circadian changes in Tb have been observed in many nocturnal and diurnal endotherms (eg Refinetti and Menaker, 1992b). Often these changes are correlated with activity (eg Brown and Refinetti, 1996) however there is evidence of other mechanisms involved (Corbit, 1970; Refinetti, 1996).

A daily rise in Tb is proposed to be due to an elevation in thermoregulatory set-point rather than simply the result of excess heat

production (Cabanac et al., 1976; Stephenson et al., 1984; Terai et al., 1985). Set-point temperature is adjustable and influenced by skin temperature and several other factors including exercise (Haight and Keatinge, 1973; Conn et al., 1990), arousal levels and inputs from extra-hypothalamic core temperature receptors (Corbit, 1970). Circadian rhythms in endogenous pyrogen (Scales and Kluger, 1987) and normal flora of the gastrointestinal tract (Kluger et al., 1990) have also been shown to mediate circadian rises in Tb in rodents. Thermoregulatory behaviour is a valuable tool in the determination of whether observed changes in Tb are due to a shift in thermal set-point (Satinoff and Henderson, 1977) or alternative mechanisms. According to Refinetti and Carlisle (1986b), temperature changes in the hypothalamus affect both autonomic and behavioural thermoregulation.

1.3 Behavioural Thermoregulation

Behaviour is one of the fundamental tools by which organisms regulate Tb and it is remarkably sensitive. As a result, behaviour is exquisitely attuned to body temperature regulation. This was originally established quantitatively in rodents using operant-based tasks as summarised by Weiss and Laties (1961).

Behavioural thermoregulation has been observed in a number of animals including protozoans (Mendelssohn, 1895; Malvin and Wood, 1992), crustaceans (eg Kivivuori, 1994), fish (Rozin and Mayer, 1961; Holland et al., 1992), reptiles (eg Sievert and Paulissen, 1996), birds (Bludgell, 1971) and various mammalian species including mice (Ogilvie and Stinson, 1966; Baldwin, 1968), rats (eg Weiss, 1957; Briese, 1985), hamsters (eg Refinetti, 1995b), ground squirrels (Refinetti, 1995a), cats (Clark and Lipton, 1974), dogs (Cabanac et al., 1970), pigs (Ingram et al., 1975), monkeys (eg Adair, 1976) and humans (eg Cabanac et al., 1976). Forms of behaviour such as huddling can also contribute to thermoregulation (particularly in young animals) and is known to exist in a number of mammals (eg Canals et al., 1989) including small marsupials (Morton, 1978; Fleming, 1980).

Behavioural thermoregulation can be measured in a number of ways. Visual observations can be made of animals in temperature gradients, animals can be trained to perform an operant task to control Ta, or the position of an animal can be continuously monitored in a temperature gradient using an automated system (Gordon et al., 1983). The latter method enables an unrestrained animal to selectively choose a Ta in accordance with physiological requirements and is the selected experimental method used in behavioural studies. The use of telemetry to assess qualitative and quantitative data in behavioural studies such as selected temperature selection is of great benefit as shown by Ishii et al. (1996).

According to Gordon (1983b), behavioural thermoregulation is more effective (or efficient) in minimising a thermal load than autonomic thermoregulation and is linked to the metabolic requirements of an animal (Gordon, 1985). Consequently, it has been acknowledged that to evaluate the full effects of thermoregulation behavioural thermoregulation must be analysed (Marques et al., 1984) preferably with simultaneous measurements of core Tb. Thus, it becomes clear that to fully understand thermoregulatory processes in any animal species (particularly endotherms) both autonomic and behavioural parameters must be considered simultaneously.

1.3a Temperature Regulation in Ectotherms

Behaviour plays an important role in thermoregulatory control in ectotherms as they generally lack internal mechanisms for temperature homeostasis. Little research exists on temperature regulation in invertebrates with most ectothermic studies concentrating on reptilian species (eg Hammel et al., 1967). However, temperature selection has been observed in protozoans (Malvin and Wood, 1992) and in the crayfish, temperature acclimation has been shown to alter temperature selection and avoidance behaviour (Kivivuori, 1994).

Ectotherms characteristically utilise their environment to achieve their selected level of Tb. This has been observed in many species, both in laboratory and field studies (eg Peterson, 1987). Eurythermality in the Northern alligator lizard (*Gerrhonotus coerulus*) is caused by variations in selected body temperature rather than fluctuations in body temperature around the particular regime body temperature (Campbell, 1985). This observation suggests that a range of temperatures may exist as the ectothermic set-point rather than a more precise set-point temperature as suggested in the endothermic thermoregulatory system.

The control of core Tb has been extensively studied in various reptilian species. Behavioural selection of Ta has been observed in lizards (Bowker, 1984; Sievert and Paulissen, 1996) and is strongly linked to photoperiod (Sievert and Hutchison, 1988) which is correlated with activity pattens (Cortes et al., 1994). However, according to Cowgell and Underwood, (1979), behavioural thermoregulation in lizards is mediated by an endogenous circadian clock and is not purely controlled by levels of activity. The correlation of activity and temperature selection in ectothermic thermoregulation is therefore unclear. It is likely that temperature and activity are not totally dependant but are somewhat related.

In lower vertebrates the pineal complex is sensitive to light and has been shown to be involved in the control of thermoregulatory behaviour (Tosini and Menaker, 1996). Thus, photoperiod does influence the selection of selected Ta. It has also been observed that both heat and light have definite but distinctive impacts on behavioural thermoregulation in lizards (Sievert and Hutchison, 1988). Consequently lizards and, possibly other ectotherms, appear to react to temperature and light in their environment in order to regulate core Tb.

Ectotherms may not only behaviourally regulate according to the thermal state of the environment. For instance, interactions between

pressure and temperature have been shown to affect behavioural preference for environmental temperature and pressure in crustaceans (Brauer et al., 1984). In addition, hypercapnia decreases selected body temperature in the toad (Branco and Wood, 1994) and during dry environmental conditions, evaporative water loss is reduced through behavioural hypothermia in the toad (Malvin and Wood, 1991). Furthermore, hermit crabs spend less time investigating potential shells while hypoxic reflecting a greater concern for energy minimisation (Côté et al., 1998). Thus, some ectotherms are able to internally react to environmental changes and adjust their thermal state accordingly facilitating survival.

Some ectothermic vertebrates utilise physiological mechanisms to achieve thermal homeostasis in similar ways to endothermic animals. Circadian rhythms of Tb are physiologically controlled in the iguana (Tosini and Menaker, 1995) and tuna are able to thermoregulate combining behavioural and physiological thermoregulation as a means of survival (Holland et al., 1992). Thus, although behaviour may be the primary mechanism of thermoregulation in ectotherms other mechanisms do exist to further refine thermogenic state.

1.3b Behavioural Thermoregulation in Endotherms

The role of behaviour in thermoregulation was originally established quantitatively in rodents such as rats as summarised by Weiss and Laties (1961). Many of these studies were operant task based and considered metabolic variables such as B group vitamins and thyroid hormones. It was established by these early studies that behaviour was exquisitely attuned to Tb regulation in mammals.

Behaviours such as huddling have been observed in small mammals as an effective behavioural response to cold temperatures (Pearson, 1960). Changes in thermoregulatory behaviour during food deprivation have also been often observed when animals are exposed to the cold (Weiss, 1957;

Hamilton, 1959; Swiergiel, 1987; Ostheim, 1992). These types of behavioural responses enable animals to conserve energy.

Birds have not been extensively studied with respect to behavioural thermoregulation and existing studies have involved the use of operant techniques resulting in species-specific observations. It has been established that there is a complex interaction of responses between behavioural and autonomic thermoregulation in birds (Horowitz et al., 1978). Generally, it appears that behavioural thermoregulation supplements autonomic thermoregulation in birds particularly when environmental temperatures are high.

Behavioural responses may be quickly acquired by birds to minimise thermal stress (Laudenslager and Hammel, 1977) and thermoregulatory behaviour has been shown to be utilised during heat stress in pigeons (Schmidt and Rautenberg, 1975; Schmidt and Simon, 1979), and in the domestic fowl (Richards, 1976; Hooper and Richards, 1991). Under cold environmental conditions however, autonomic responses appear to be of more importance in avian thermoregulation (Hooper and Richards, 1991) unless there is a shortage of food. Fasted pigeons perform operant cold-escape tasks (heat gain) at higher rates than normally fed animals (Ostheim, 1992)

Measuring Tb and the selected Ta over a 24 hour period is of paramount importance in understanding how the modulation in set-point is involved in the circadian control of Tb. Humans and rodents have been shown to facilitate autonomic and behavioural responses to produce a daily rise in core Tb (Cabanac et al., 1976; Stephenson et al., 1984; Szymusiak et al., 1985; Scales and Kluger, 1987). This indicates that the daily rhythm of Tb is an integral part of thermal homeostasis and the daily temperature rise corresponds to a regulated elevation in the thermal set-point (Hensel, 1981; Kluger, 1991). In the nocturnally active rat it has also been suggested that

the elevation in core Tb at night is mediated by an elevation in set-point (Kluger, 1991; Scales and Kluger, 1987).

Shifts in endothermic set-point of thermoregulation have often been argued as the basis of changes in regulated Tb observed in mammals when exposed to hypoxia (low O_2) (see Wood, 1991) or bacterial endotoxin (ie fever) (see Kluger, 1986). Briese (1985) was the first to provide evidence contrary to this belief when he demonstrated that rats select Ta 195° out of phase with hypothalamic temperature circadian variation. Kluger (1991) pointed out some methodical flaws in Briese's study yet other studies have supported Briese's results. Refinetti (1995b) found Tb and selected Ta to be 180° out of phase in the golden hamster and Gordon (1994) suggested that behaviour has little if any role in circadian elevations in Tb.

The proposal that circadian rhythms of Tb are due to a shift in set-point implies that the phase of elevated Tb is equivalent to a minor febrile episode and during this phase, warmer environments would be sought by an organism together with an increase in heat production and conservation. Periods of the day in which Tb is low would result in an animal seeking warmer environments and vice versa for periods of high Tb. However, Briese (1985) was unable to conclusively demonstrate that rhythmic shifts in set-point are reflected by selected temperature of Wistar rats (behavioural thermoregulation). Briese (1985) concluded that the set-point for behavioural rhythms does not shift with circadian rhythm although autonomic responses do have a built-in cyclical nycthemeral shift. Similarly, Refinetti (1998) found that the Tb rhythm of gerbils was not caused by a rhythmic oscillation of the thermoregulatory set-point. The nycthemeral cyclic change in body temperature in humans has been proposed to be due to a nycthemeral cyclic change in the set point near to which body temperature is kept by both behavioural and autonomic thermoregulatory responses (Cabanac et al., 1976).

Lower energy requirements for behavioural thermoregulation make it the selected effector mechanism for thermal homeostasis in birds (eg Schmidt, 1978) and mammals (eg Gordon, 1983b). However, it has been shown that rats select cooler Tas during nocturnal hours (Briese, 1985; 1986) and Ta selection and Tb rhythms have been observed to be out of phase in the golden hamster (Refinetti, 1995a). It has been established in the rat that autonomic effectors control the elevation in Tb during the onset of the dark phase with behavioural effectors having little if any role (Gordon, 1994). However, behavioural thermoregulation plays a vital role in allowing the avoidance of excess elevations in nocturnal core Tb and facilitating the recovery of diurnal core Tb (Gordon, 1994). Thus, although behavioural thermoregulation may not be the key effector in mammalian Tb control its effects must be considered.

Although experimental evidence is presently limited, Refinetti (1995b) has suggested that only nocturnal endotherms exhibit a daily rhythm of temperature selection. Rhythms of Ta selection have been observed in the nocturnal rat (Briese, 1985; 1986; Gordon, 1993b; 1994) and in the nocturnal hamster, *Mesocricetus auratus* (Gordon, 1993b; Refinetti, 1995a; 1995b) but not in the diurnal ground squirrel (Refinetti, 1995b). Thus, further studies of Ta selection in diurnal and nocturnal endotherms are required. In addition, due to the controversy surrounding the impact and role of thermoregulatory behaviour on daily Tb rhythms it is obvious that further studies are warranted in this area.

1.3c Behavioural Thermoregulation in Marsupials

The importance of behavioural thermoregulation is yet to be significantly investigated in marsupial species. Thermoregulatory behaviour in the form of huddling has been observed in some small marsupials such as *Sminthopsis crassicaudata* (Morton, 1978) but has been found to be of little thermoregulatory significance in other small marsupials such as the feathertail glider, *Acrobates pygmaeus* (Frey and Fleming, 1984).

Fleming (1980), however demonstrates significant energetic advantages of huddling in *P. breviceps*. Increasing evaporative cooling by licking fur, reducing activity and changing posture have all been observed in marsupials as behavioural adaptations to high temperatures (Robinson and Morrison, 1957). Changes in posture to reduce surface area have also been observed in young *Didephis virginiana* (Morrison and Petajan, 1962) and *P. breviceps* (Holloway and Geiser, 2000).

Reducing core Tb (ie entering torpor) when environmental temperatures become unfavourable is known to occur in small marsupials (Bartholomew and Hudson, 1962). Torpor has been reported in small marsupial species and may occur on a strict daily pattern (eg Geiser and Masters, 1994) or be deep and prolonged (Geiser, 1986). Preferred Ta during torpor and homothermia has been investigated in *Sminthopsis macroura* indicating a preference for higher Ta near the TNZ with food regimes having little effect (Song et al., 1998). This indicates that this marsupial species utilises Ta to reduce energy costs. Sleep, estivation and torpor are also thought to influence core Tb in *P.breviceps* (Fleming, 1980; Dawson and May, 1984). Torpor is essential to survival in small species as it minimises energy loss as reported in *P. breviceps* by Kortner and Geiser, (2000).

This study aims to address behavioural thermoregulation in a small marsupial species by investigating circadian rhythms of selected Tas with simultaneous measurements of core Tb in *M. domestica.*

1.4 Autonomic Thermoregulation in Endotherms

Birds and mammals are equipped with many physiological processes in additional to behavioural means to enable them to maintain a constant core Tb (George, 1984). These include the acquisition of an insulative coat, involuntary muscle contractions (shivering), voluntary muscle contractions (exercise and locomotion), vasomotor control, and non-shivering

thermogenic mechanisms. All of these processes involve increasing cellular and tissue metabolism to produce heat. Shivering and non-shivering thermogenesis (NST) increase oxygen consumption and heat production providing partial homeostasis of normal body temperature in neonates and complete homeostasis in adults. In this classical scheme, metabolism is maintained at a basal level over a range of ambient temperatures called the thermoneutral zone (TNZ). In adults, body temperature is maintained at ambient temperatures below the TNZ by thermogenic responses. Mechanisms such as evaporation through sweating and respiration also exist to facilitate heat loss (Robinson and Morrison, 1957) and humoral and hormonal influences contribute markedly to the maintenance of a relatively constant internal core Tb in mammals and birds.

Exercise, activity and shivering thermogenesis all involve noticeably increased skeletal muscle activity and have been observed in many avian and mammalian species (eg, Prinzinger et al., 1991). Birds and eutherian mammals can also increase their thermogenic capabilities through an increase in NST (Jansky, 1995) which does not involve obvious muscle contractions. NST is apparent in hibernating and newborn mammals, can be induced in adult mammals by cold adaptation and is regulated via hypothalamic controls (Jansky, 1973). In these mammals, NST is based primarily on noradrenaline released from sympathetic nerve endings. In eutherian mammals, NST is localised mainly in skeletal muscle and brown adipose tissue (BAT) with small amounts generated from the liver, intestine, heart and brain (Jansky, 1973).

Thermogenesis is also controlled humorally by diet and endogenous compounds such as adrenalin, thyroid hormones, glucocorticoids, glucagon and various peptides and steroids (Jansky, 1995). Of these, thyroid hormones are widely accepted as a major contributor to mammalian thermogenesis.

1.4a Circadian Rhythms of Body Temperature in Endotherms

Core Tb is influenced by light and dark phases leading to the establishment of a circadian pattern of core Tb in endotherms. Circadian patterns of Tb are evident in nocturnal and diurnal species and in both birds and mammals, the range of daily fluctuations in Tb decrease with increasing body mass (Prinzinger et al., 1991). As reviewed by Refinetti and Menaker, (1992b) the circadian rhythm of Tb is modulated by a number of factors including activity, thermogenic mechanisms and disease.

Circadian rhythms of body temperature and oxygen consumption show concurrent changes with activity levels in both diurnal and nocturnal animals (Aschoff, 1970) indicating a strong causal relationship between body temperature and activity rhythms. This was first established by Chossat (1843) in starved doves and later by Jurgensen (1873) in man. Kleitman (1923) then demonstrated that sleep deprivation fails to abolish body temperature rhythms in humans.

Birds show a typical rhythm amplitude of 0.8 to 3.7°C in a 24 hour cycle of core Tb (Refinetti and Menaker, 1992b). Circannual variations in core Tb are also observed in many birds as a result of seasonal gonadal activity (Prinzinger et al., 1991). Hormones such as melatonin also influence the circadian rhythm of Tb in birds (eg Binkley et al., 1971).

Circadian control of core Tb has been extensively studied in mammalian species. Nychthemeral variations in Tb are species-specific (eg Bligh and Harthoorn, 1965) with great variations in peaks of Tb throughout a 24 hour cycle. Both diurnal and nocturnal species have been studied and in rodents the only difference between the two groups was the acrophase (peak temperature) which occurred during the day in diurnal species and at night in nocturnal species (Refinetti, 1996).

Both monophasic and biphasic circadian rhythms of Tb have been observed in mammalian species. For example, Pickard et al., (1984) observed a splitting of the circadian Tb pattern in hamsters and a bimodal pattern of Tb has been observed in the tree shrew (Refinetti and Menaker, 1992a). However, the rat displays a single peak in Tb throughout a 24 hour cycle (eg Gordon, 1993a) not unlike many other mammalian species. These differences in circadian patterns between mammalian species have not yet been justified.

1.4b Autonomic Thermoregulation in Marsupials

Marsupials have a resting minimum metabolic rate within thermoneutrality (basal metabolism) that is 70% of the corresponding eutherian level (Kleiber, 1971) at any body mass (eg. Dawson and Hulbert, 1970). This was noted by Martin (1903) who was the first to compare the metabolism of monotremes, marsupials and eutherian mammals. In addition, marsupials tend to have lower Tbs than eutherians (eg., MacMillen and Nelson, 1969). However, below thermoneutrality, metabolic rates are equivalent in both taxa (Hinds and MacMillen, 1984). Despite these lower levels of thermoregulation in comparison to eutherians, marsupials do possess characteristics which demonstrate that they are excellent endotherms (Dawson, 1973; Hudson and Dawson, 1975; Dawson, 1984).

Many aspects of thermoregulation in marsupials have been investigated involving both continuous and non-continuous measures of core Tbs (eg Morrison, 1965; Dawson and Hulbert, 1970; Halse and Rose, 1988; Kortner et al., 1998) and pouch temperatures (eg Morrison and Petajan, 1962; Shield, 1966; Gemmell et al., 1987). At birth marsupials are small, naked, ectothermic and extremely immature in many physiological systems. Thermoregulatory development has been proposed to involve the ability to respond to fluctuating temperatures, the onset of thyroid activity and the ability to attain a steady adult basal core temperature (Hulbert, 1988). Adult marsupials exhibit comparable Tbs to eutherian mammals despite their

lower standard metabolic rates. Mechanisms of heat production, however, often appear different to that of the classical eutherian pattern (eg Reynolds and Hulbert, 1982).

According to Hayward and Lisson (1992) marsupials lack BAT and thus do not appear to have BAT based NST. The proposal that marsupials lack BAT has been used to explain the slow arousal rates observed in marsupials (Wallis, 1979). Despite their apparent lack of BAT, *P. Breviceps* are able to tolerate extremely low Ta (Holloway and Geiser, 2001a; 2001b). Noradrenaline mediated NST in adult marsupials appears to be absent or of little importance (Nicol, 1978; Wallis, 1979; Reynolds and Hulbert, 1982; Dawson and Olson, 1988; Opazo et al., 1999). NST however may occur in marsupials due to the actions of endocrine rather than nervous stimulation (Smith and Dawson, 1985) and it has been proposed that noradrenaline-mediated NST is found only in macropod marsupials (Nicol et al., 1997; Rose et al., 1999). Furthermore, skeletal muscle is propsed to be the site of NST in *B. gaimardi* (Ye et al., 1995). The role of NST in marsupial thermoregulation therefore remains unclear.

Many existing studies on thermoregulation in marsupials fail to address circadian variations in core Tb (eg Dawson et al., 1969). As mammals typically display Tb fluctuations throughout the day the ignorance of these circadian variations may have significant manifestations in the assessment of some thermoregulatory parameters. Circadian Tb rhythms in marsupials were first measured in seven species of dasyurids by Morrison (1965). Circadian patterns of Tb have also been recorded in the Tasmanian devil, *Sarcophilus harrisii* (Morrison, 1965; Guiler and Heddle, 1974), kangaroos (Brown and Dawson, 1977), the common wombat, *Vombatus ursinus* (Peters and Rose, 1979), the American opossum, *Didelphis virginiana* (Treagust et al., 1980), the sugar glider, *Petaurus breviceps* (Dawson and May, 1984), the brush-tailed possum, *Trichosurus vulpecula* (Halse and Rose, 1988; Gemmell and Cepon, 1993), the grey short-tailed opossum, *Monodelphis domestica* (Rivkees and Reppert, 1990), the Tasmanian bettong,

Bettongia gaimardi (Rose et al., 1990) and the quoll, *Dasyurinus viverinus* (Jones et al., 1997). These rhythms are usually comparable to those observed in nocturnal eutherian species, although actual Tbs observed are lower. Previous studies haves also investigated core Tb rhythms in the two monotremes; the platypus, *Ornithorhyncus anatinus* (Grigg et al., 1992) and the echidna, *Tachyglossus aculeatus* (eg Grigg et al., 1989).

As fluctuations in marsupial Tb can be quite high throughout a 24 hour period, in this study I have measured all Tbs continuously via radiotelemetry to prevent sampling errors due to circadian differences.

1.5 Thyroid Activity and Thermoregulation

The function of thyroid hormones in thermogenesis is a well studied area as reviewed by Freake and Oppenheimer (1995) and Hulbert (2000). The thyroid hormones have widespread effects on the body with their principal actions being stimulation of metabolic rate and promoting growth. The thyroid also functions in other areas including regulation of cardiac output, sympathetic nerve activity, reproductive and neural function, and early development, including amphibian metamorphosis (Eayrs, 1966; Ford, 1968; Fisher et al., 1977; Galton, 1983; Schwartz, 1983; Slotkin and Sleptis, 1984).

The regulation of thyroid hormone secretion occurs at various levels but depends primarily on a feedback system between the anterior pituitary which releases thyroid-stimulating hormone (TSH) and the thyroid gland which releases thyroxine (T_4) and triiodothyronine (T_3). The main positive regulator is TSH and the thyroid hormones act as negative regulators (Shupnik et al., 1989). T_4 is the principal hormone and through iodination may convert to T_3. Both thyroid hormones are usually transported in the blood bound to plasma protein (particularly albumin) but may also occur free in the plasma. Once reaching target cells, T_3 and T_4 bind to cell membrane receptors altering the internal metabolic state of the cell.

Thyroid hormones affect metabolism and thermogenesis stimulating heat production and thus metabolic activity. Thyroidectomy in both eutherian mammals and marsupials causes a significant reduction in metabolic rate (Hulbert and Augee, 1982).

The hormonal control of cold-induced thermogenesis is modulated by thyroid hormones. During acute cold exposure a typical endothermic response involves an increase in thyroid activity and thyroid hormone metabolism. According to Dauncey (1990), there is no clear evidence linking elevations in thyroid hormone metabolism with short-term cold exposure. However, thyroid hormones do play a significant role in the NST component of cold adaptation (Guernsey and Edelman, 1983; Silva, 1995) and are utilised during hibernation (Hulbert, 1978; Nicol et al., 2000). As a hormonal mechanism, T_3 and T_4 are therefore utilised during extended exposures to cold temperatures. The thermogenic effect of the thyroid hormones is dependent on animal size, species and the environment (Yousef and Johnson, 1975; Guernsey and Edelman, 1983). In addition, utilisation rates of T_3 and T_4 correlate with predictions made on energy balance requirements. For example, utilisation rates increase with cold exposure and regular physical activity and decrease with heat exposure, age and during fasting (Tomasi, 1991).

1.5a Eutherians

In mammalian species, the primary function of the thyroid gland is the control of basal metabolism and thermogenesis. In response to cold exposure, TSH is released. The subsequent release of T_4 into the bloodstream causes an increased rate of metabolism in many tissues throughout the body (Edelman, 1974). This is an essential part of survival (Sellers and You, 1950).

Variations in metabolism are controlled by the thyroid gland to achieve thermal homeostasis in mammals (Whitaker et al., 1990; Tomasi,

1991) and circadian variations of thyroid hormones exist. Thyroid hormones, particularly T_4, act on activity rhythms in rodents (eg McEachron et al., 1993) and the circadian system has been shown to be modulated by thyroid hormones in the hamster (Beasley and Nelson, 1982) and the rat (Vessotskie et al., 1993). Thus, circadian patterns of Tb observed in mammals occur due to circadian alterations in thyroid hormone levels. Such rhythms have been recorded in rats (Cokelaere et al., 1996) and in humans (Fisher, 1996) and have also been observed by Shido et al., (1993) who found that heat exposure at fixed time periods altered day-night variations of plasma thyroid hormones in rats. According to Cokelaere et al., (1996), feeding patterns can also influence circadian rhythms of plasma thyroid hormone levels in rats.

1.5b Marsupials

There is very little literature on the thyroid status of marsupials in comparison to other mammals with most existing studies concentrating on the development of thyroid activity in pouch young (eg Hulbert, 1988). However, thyroid hormone levels have been measured in a few adult marsupial species (eg Hulbert, 2000). Albumin is a common thyroxine-binding plasma protein found in eutherians and marsupials as well as a number of other animal species (Richardson et al., 1994). Thus, similar techniques can be used to measure thyroid hormones in eutherians and marsupials.

Thyroid activity in adult marsupials is relatively similar to that in eutherian mammals (Hulbert and Augee, 1982). Total T_4 concentrations in serum have been found to be greater in the eutherian rabbit and rat, than in the marsupial bandicoot with free T_4 levels similar between the three species (Hulbert and Augee, 1982). Lower total T_4 levels have also been observed in *P. tridactylus* with free levels equivalent to the normal human value (Nicol, 1977) and low T_4 levels have been measured in the koala (*Phascolarctus cinereus*) (Lawson et al., 1996). It is important to remember that total thyroid

hormone levels measured in a species reflect the thyroid hormone-binding plasma proteins (ie. their concentrations in the plasma and their affinity for T_4 and T_3).

T_3 levels however have not been measured extensively in marsupials and existing studies do not indicate any substantial difference between eutherian and marsupial species (Hulbert, 2000). Total and free T3 levels have been measured in *M. eugenii* (Janssens et al., 1990) and *P. cinereus* (Lawson et al., 1996) however only total T3 levels have been measured in *I. macrourus* (Hulbert and Augee, 1982)

As in eutherian mammals, the environment plays a role in thyroid hormone levels in marsupials and a seasonal pattern of thyroid activity has been observed in *M. eugenii* (Kaethner and Good, 1975). A large increase in thyroid activity has been observed in *A. stuartii* following cold acclimation (Withers and Hulbert, 1988) indicating a relationship between thyroid activity and cold-induced increases in metabolic rate in marsupials. Obviously, due to the lack of literature available on thyroid activity in marsupials, more studies are warranted to understand the role of the thyroid gland in the thermal homeostasis of marsupials.

1.6 Thermoregulation and Hypoxia

Exposure to hypoxic conditions (ie lowered ambient oxygen concentrations) initiates a variety of physiological and behavioural mechanisms in various species (Brauer et al., 1986; Wood, 1991). General thermoregulatory responses to hypoxia have been assessed in many animal species with most studies concentrating on reptiles and rodents.

When exposed to lower ambient O_2 concentrations (ie., hypoxia), animals typically exhibit a reduction in core Tb. This reduction is generally hypothesised to be due to a functional shift in the set-point of the thermoregulatory system to reduce oxygen requirements during times of

limited oxygen supply (Wood, 1991). There is substantial evidence that the lowering of the thermoregulatory set-point during hypoxia occurs via a direct action on central neural structures (Gautier, 1996).

Armadillos increase their metabolic rate in response to hypoxia (Frappell et al., 1992) as do echidnas (Frappell et al., 1994) and ponies (Korducki et al., 1994). This is contrary to observations in other animal species in which hypothermia is a key hypoxic response. In fact, hypothermic responses to hypoxia are a taxonomically widespread phenomenon occurring in both water- and air-breathing animals (Frappell et al., 1992). This hypothermic response is a fundamental tool of survival when oxygen supply is limited for cellular metabolism.

1.6a Ectotherms

The metabolic and physiological responses to hypoxia in ectothermic invertebrates have been reviewed (Kluger, 1991; Greishaber et al., 1994). In addition, thermal changes in response to hypoxia in ectothermic vertebrates have been observed, particularly in lizards (eg Kluger et a., 1975; Deen and Hutchison, 2001).

Ectotherms have been shown to induce a shift in thermal set-point when exposed to hypoxic conditions (Hicks and Wood, 1985). This indicates that metabolism is being altered to accommodate the lack of oxygen available in the environment. Hypoxia-induced thermoregulatory changes observed in ectotherms are both neural (including behavioural selection of Tb) and physiological (Hicks and Wood, 1985). These generalised responses provide an effective adaptation to hypoxia and thus promote survival.

Behavioural temperature regulation during hypoxia appears to be the selected mechanism among many ectotherms (Dupré and Wood, 1988). When exposed to a hypoxic environment lizards show a preference for lower Tas and thus Tb is lowered (Kluger et al., 1975; Hicks and Wood,

1985). This behavioural hypothermia in lizards is regarded to be a regulated response to hypoxia (Hicks and Wood, 1985). Similarly, protozoans (i.e. Paramecia) select lower temperatures in a thermal gradient under hypoxic conditions (Malvin and Wood, 1992). Thus, even unicellular animals lacking a nervous system exhibit a behavioural hypothermic response to hypoxia.

1.6b Eutherians

It has been shown that hypoxia significantly reduces metabolic rate in endotherms (Dupré et al., 1988) and that rodents select significantly lower Tas during hypoxia while concurrently exhibiting a hypothermic response to hypoxia (Gordon and Fogelson, 1991; Dupré and Owen, 1992). These studies support the hypothesis that hypoxia shifts the set-point for Tb control to a lower level in eutherian mammals. However, not all mammals show a typical hypothermic response to hypoxic conditions. For example, acute hypoxia does not depress metabolic rate in ponies as it does in small mammals (Korducki et al., 1994).

Typically though, in response to hypoxia, mammals exhibit an increase in ventilation and cardiac output (to increase O_2 supply) together with a reduction in Tb (to reduce O_2 demand) and an alteration of thermoregulatory behaviour (Wood, 1991). To maintain Tb , ectotherms typically utilise behavioural mechanisms while endotherms use not only behavioural strategies but also require physiological mechanisms when the Ta is either above or below the TNZ. Hypoxic mammals demonstrate heat loss from peripheral vasodilation, behavioural hypothermia (ie selection of lower Tas to accelerate heat loss), and reduced heat production (blood is redistributed away from brown fat regions) (Wood, 1991). Therefore, in mammals it is assumed that physiological and behavioural mechanisms act in unison to lower Tb in response to hypoxia.

Rodents have been shown to us behaviour to regulate a lower Tb during hypoxia exposure (Gordon and Fogelson, 1991; Dupré and Owen, 1992) in a pattern that closely parallels the thermoregulatory behaviour observed in ectotherms under similar conditions (Hicks and Wood, 1985). However, the behavioural effect of hypoxia on many mammals has not yet been determined.

1.6c Marsupials

There is limited literature on the effects of hypoxia on thermal homeostasis in marsupials. Hypoxia has been shown to reduce metabolic rate in some small marsupial species (Frappell et al., 1992). Whether this is the result of a shift in the set-point for thermoregulatory control or is due to some other thermoregulatory mechanism is not apparent. Hypoxic effects on Tb rhythms and behavioural selection of selected Ta in marsupials have not been determined. This study has therefore endeavoured to determine hypoxic effects on Tb rhythms and selected Ta in *M. domestica* and one *P. breviceps*.

1.7 Fever and Thermoregulation

Fever is defined as an abnormal elevation in Tb or an elevation in the level around which Tb is regulated. Febrile responses have been well studied in many mammalian species, particularly rodents, and mechanisms of fever have been extensively reviewed (eg Hellon, 1978; Kluger, 1991).

Fever occurs due to bacterial or viral infection and can be induced by a number of exogenous and endogenous pyrogens (fever-producing agents). Pyrogens usually induce peripheral vasoconstriction and shivering followed by a concomitant elevation in core body temperature (eg Rosendorff and Mooney, 1971). Various studies have looked at the effects of different pyrogens on thermoregulation in a number of different species, however, most studies have concentrated on bacteria-induced fever. The febrile

response to bacterial endotoxin has been recognised as a means of survival in both ectothermic and endothermic animals (Kluger et al., 1975) increasing the resistance of the host to the bacterial pathogen.

In humans, Tb changes during fever reflect a change in set point rather than a change in the gain of the thermoregulatory processes (Cabanac and Massonet, 1974). This implies that warm sensors become less responsive and cold sensors more responsive to temperature changes during fever as postulated by Mitchell et al., (1970).

Induced fever appears to have a reduced effect in aged animals as observed in aged humans (Collins et al., 1977), squirrel monkeys (Clark et al., 1980), New Zealand white rabbits (Lipton and Ticknor, 1979; Ferguson et al., 1981; Naylor et al., 1985) and rats (Maitland et al., 1985; Shemi and Kaplanski, 1995). The inability of aged animals to conserve and generate heat may contribute to a reduced febrile response (Naylor et al., 1985). This indicates that the effector mechanisms of the thermoregulatory system become inefficient with age. Alternatively, alterations to central sensitivity to pyrogen (Clark et al., 1980) or an inability to respond effectively to the cold (Collins et al., 1977) may explain the different reactions to fever in young and old animals.

1.7a Ectotherms

Generally, ectotherms display behavioural hyperthermia in response to prostaglandins (eg Hutchison and Erskine, 1981) and bacterial endotoxin (eg Reynolds et al., 1976). This behavioural fever is probably a survival mechanism although it is not always observed in response to all pathogens and parasites (eg Adamo, 1998). In addition, metabolic rates have been shown to increase significantly during fever in ectotherms (Muchlinski, 1985).

Febrile responses are not observed in all ectotherms. For example, snails do not develop a fever in response to a variety of pyrogens including bacterial endotoxin, killed bacteria, interleukin or prostaglandin (Cabanac and Rossetti, 1987). As a result, Cabanac and Rossetti, (1987) suggest that fever appeared after the emergence of molluscs yet before that of the arthropods in the course of evolution.

Frogs (*Rana esculenta*) behaviourally select warmer water when injected with killed pathogenic bacteria accompanied with an increase in colonic temperature (Myhre et al., 1977). In lizards, an elevation in Tb during fever enhances survival (Kluger et al., 1975) and behavioural fever is triggered by pyrogen action on centres other than those affected by the pineal gland manifesting an elevation in the central thermostat (Firth et al., 1980). Thus, the initiation of behavioural fever in ectotherms may not be mediated by areas specifically involved in normal behavioural thermoregulation.

1.7b Endotherms

Bacterial endotoxin from *Eschericia coli* (*E.coli*) is known to produce a febrile response in many mammalian species (eg Kozak et al., 1994; Severinsen and Øritsland, 1991) and febrile responses have been observed in avian species (D'Alecy and Kluger, 1975; Maloney and Gray, 1998). However, there is controversy in the literature as to whether or not rats produce fever when injected with endotoxin. For example, normal Sprague-Dawley rats have been shown to display a hypothermic response to intravenous *E.coli* endotoxin while Brattleboro rats become febrile to the same endotoxin (Stitt and Shamada, 1987). Hypothermic responses to bacterial LPS in rats have also been observed by Derijk et al., (1994) and Romanovsky et al., (1996). Reactions to bacterial pyrogens are therefore not only species-specific but differences within a species may also exist although these differences may be dose related.

Fever-induced (LPS) changes in Tb are dose-dependant in some mammalian species such as mice (Kozak et al., 1994). Thus, febrile responses are sometimes not reported due to the time involved in the induction of the response. It is therefore of importance to continuously monitor Tb of an animal under febrile conditions.

Febrile responses in mammals have been found to be monophasic (eg Conrad et al., 1997) or biphasic (eg Soszynski et al., 1991) while a monophasic pattern is characteristic of avian fever (Maloney and Gray, 1998). Romanovsky et al (1996) suggests that at low doses of pyrogen mammals typically develop a monophasic rise in Tb, at moderate doses a biphasic fever and at high doses hypothermia is observed as septic shock occurs. However, in some mammalian species such as mice only biphasic rises in Tb are observed in response to various doses of exogenous pyrogen (LPS) (eg Kozak et al., 1994).

Many studies have looked at short-term effects of endotoxin on thermoregulation with febrile responses observed as brief rises in core Tb with dosage a possible critical factor (Morimoto et al., 1986). Prolonged fever has also been observed in rats after a single injection of *E.coli* endotoxin (Severinsen and Øritsland, 1991). This prolonged effect suggests that endotoxin may increase the thermal set-point in the mammalian thermoregulatory system.

Behavioural adaptations to fever have also been observed in mammals. While febrile, Mongolian gerbils choose lower environmental temperatures and increase Tb (Akins and Thiessen, 1990). Newborn rabbits when injected with bacterial endotoxin initially select warmer temperatures with cooler temperatures subsequently selected in accordance with changes in core Tb (Szekely, 1984). These studies suggest that increases in Tb in mammals due to pyrogens are somewhat limited by behavioural selection of lower Tas although initial phases of fever are accentuated by preferences for warmer Tas. Satinoff and Henderson (1977) reported that the height of a

fever is determined to some extent by behavioural selection of Ta. It can consequently be assumed that fever may play a role in the mammalian febrile responses although more studies are obviously required to validate this.

There is substantial evidence that variations in febrile responses occur among different eutherian species. Fever as a hyperthermic response to bacterial endotoxin is yet to be investigated in marsupials or monotremes to determine further mammalian differences. As a result, this study has investigated whether a febrile response to *E. coli* is present or absent in *M. domestica* and whether a behavioural fever has any role in this marsupial species

1.8 Outline of the study

In the present study, aspects of endothermy are investigated in the South American grey short-tailed opossum (*Monodelphis domestica*) and one Australian sugar glider (*Petaurus breviceps*). *M. domestica* and *P. breviceps* are small members of the marsupial family weighing 90 to 130g and 80 to 150g respectively (personal observations). A summary of some of the characteristics of these species is listed in Appendix 1. Although only one *P. breviceps* was used in this study (due to difficulties in obtaining animals of this species, see Preface and Chapter 2 Material and Methods), some of the results from this single animal contrasted so strongly with the laboratory-bred *M. domestica* it was of interest to include the results from this animal for comparative reasons.

Previous studies of thermoregulation in marsupials have often failed to address circadian variations in core Tb under varying conditions although normal circadian patterns have been verified in a number of species. In addition, behavioural thermoregulation has not been previously investigated in marsupials to determine the role of Ta selection in thermal homeostasis.

In this study I have determined circadian patterns of Tb in:

- adult *M. domestica*
- juvenile *M. domestica*

Comparisons were made between circadian rhythms observed in juvenile and adult animals with respect to core body temperature. It was hypothesised that Tb rhythms will occur in adult laboratory-bred *M. domestica* and that daily core Tb would be comparable between adults and juveniles with different circadian patterns observed amongst different age groups. Thermoregulation in the cold and thyroid hormone levels were also investigated in *M. domestica*.

In this study I have also determined the circadian variations in Ta preference while in a longitudinal thermal gradient and the effects of Ta selection on core Tb in adult *M. domestica*. It was hypothesised that *M. domestica* would select Tas in phase with their circadian variations of Tbs and metabolic rate.

This study also investigated the following in laboratory-bred *M. domestica* and one *P. breviceps* obtained from its natural habitat:

- the effects of bacterial endotoxin (LPS) on thermoregulatory responses (ie circadian patterns of core Tb and selected Ta)
- the effects of hypoxia on thermoregulatory responses
- the effects of a combination of LPS and hypoxia on thermoregulatory responses.

Under hypoxic conditions it was proposed that thermoregulatory responses would be lowered and when injected with LPS, core Tb would be significantly increased with simultaneous preferences for warmer Tas. During a combination of LPS exposure and hypoxia it was hypothesised that the true effects of LPS exposure and hypoxia would each cancel out. As a consequence, normal rhythms of Tb and normal patterns of Ta selection would be observed rather than a hyperthermic effect (typical of LPS

exposure) or a hypothermic effect (typical of hypoxia). This would support the set-point theory of thermoregulation.

The significance of this study is to further our knowledge of thermoregulatory strategies in marsupials which will enhance our understanding of the ecological niches occupied by various species. There are no published data on fever in marsupials and the effects of hypoxia and thermoregulatory behaviour on thermoregulation in marsupials have not been determined. Such information may assist in the understanding of the impact and role of these parameters on daily Tb rhythms in mammals generally. The study also aimed to determine whether animals bred and maintained in a laboratory environment thermoregulate in a similar or different manner to a wild-bred species of a similar size.

It is hoped that this study may provide some insight into the widely accepted set-point theory of thermoregulation. As a comparative study, it may also contribute significantly to our knowledge of the evolution of thermogenic processes.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental Animals

A colony of South American grey short-tailed opossums (*Monodelphis domestica*) and a sugar glider (*Petaurus breviceps*) were used in this study. The *M. domestica* colony was originally established in Southampton, England and was maintained at The University of Tasmania Central Animal House. The animals used from this colony were mainly older males and were sacrificed by cardiac puncture at the completion of the experiments to satisfy Australian quarantine regulations. The single *P. breviceps* was captured in forest areas in Northern Tasmania and maintained in indoor enclosures and under experimental conditions for a period of eight weeks before being released at the original capture site.

2.1a *Monodelphis domestica*

The opossums were held in plastic cages with metal grid lids containing a plastic nesting box filled with shredded paper. The cages were kept in a fully enclosed windowless room with a 14L:10D photoperiod at a Ta ranging between 28-30°C. The opossums were fed ad libitum on a special combination diet of tinned cat food, peanut oil, high protein cereal, meat meal, Veanavite, Avi-drops, Whiskettes, bananas and mealworms and water was always freely available. Animals were regularly monitored by animal house staff on a daily basis. At the completion of the experimental period, animals were sacrificed.

2.1b *Petaurus breviceps*

Suitable trapping areas in Northern Tasmania were determined through consultation with Ron Mawbey, School of Zoology, University of Tasmania who has records of sugar gliders trapped throughout Tasmania. Capture sites were located and surveyed for up to eighteen months in

sclerophyll forests with high density *Acacia, Banksia* and *Eucalyptus* species which correlated with previous locations of this marsupial species in its natural habitat (Rounsevell et al., 1991). Preliminary surveys of chosen sites, using local knowledge and traps baited but not set for actual trapping of an animal, indicated the presence of sugar gliders in the chosen area. When the time came to collect animals for experimental work, despite a total of 112 trap nights (four to six traps set each night) over a total period of ten months, only one animal was trapped. This was attributed to the increasing effects of human habituation in the trapping area and the poor weather during the majority of the active trapping period.

The sugar glider was captured using traps reported to be relatively unstressful to the animals (Mawbey, 1989). The sugar glider was held in an indoor cage (constructed of melamine board and chicken wire with a gumleaf-lined floor and containing fresh tree branches and a wooden nesting box lined with fresh gum leaves) in a fully enclosed windowless room with a 12L:12D photoperiod at a Ta ranging between 18-23°C. The animal was fed on a combination omnivorous diet provided by the Department of Zoology, University of Tasmania (Ron Mawbey, personal communication). This diet included a protein mix (honey, glucodin, complan, bonox, Horlicks, digestelact and water) or a weetbix mix (honey, water, pentavite, high protein cereal and weetbix) complemented with mince, bread, mealworms, peanut paste, sultanas, oats, pine nuts, peanuts, sunflower seeds and fresh fruit. Water was available ad libitum. The animal was released at the capture site at the completion of the study.

2.2 Determination of Circadian Rhythms of Core Body Temperature

Circadian patterns of Tb regulation were determined from transmitters implanted into the abdominal region of each animal. Circadian rhythms were determined for one adult *P. breviceps* and eighteen adult and juvenile *M. domestica.*

2.2a Surgical Procedure

M. domestica were anaesthetised with 1.5% fluorothane at 100-300 ml/min, depending upon the size of the animal, for the surgical procedure of implantation. Halothane has previously been shown to be the most effective anaesthetic in this species (Keller et al., 1988) although animals must be monitored carefully (Robinson and VandeBerg, 1994) as induction often occurs suddenly (Fadem et al., 1982). *P. breviceps* were anaesthetised with 4% fluorothane at 400ml/min or 2.5-3% isofluorane at 300ml/min for the surgical implantation of the temperature-sensitive transmitter. The latter form of anaesthesia was used for *P .breviceps* as lower concentrations could be used and it is a selected form of anaesthesia for small mammals (Eileen Wronski, personal communication). It is also a highly effective form of anaesthesia with a reduced induction and recovery time in comparison to halothane anaesthesia (personal observations).

A temperature-sensitive transmitter was implanted into the abdominal cavity via a midline incision in the abdomen. Animals were carefully monitored throughout the procedure. Immediately after the surgical procedure, animals were returned to their respective cages for a recovery period of at least 24 hours before experimental recordings began.

2.2b Recordings of Core Body Temperatures using Implanted Transmitters

The transmitters used in this study were supplied by Telonics Electronics, Mesa, Arizona, U.S.A. and Titley Electronics, Ballina, N.S.W., Australia. Telonics transmitters (CHP-IMP with S2 temperature sensors) weighed approximately 10g and were only used in adult animals. Titley transmitters with batteries were coated with a high melting point wax and weighed 1-3g; these transmitters were implanted into juvenile animals. The transmitters were calibrated with a certified mercury thermometer prior to implantation. Calibration involved using a temperature control bath to decode beeps from transmitters at temperatures ranging from 20°C to 40°C and translating the beeps into temperature recordings. Transmitters

recalibrated by the same method where possible after removal from the animal.

Signals were received from implanted transmitters by a Yaesu FRG-9600 receiver system (Dick Smith Electronics) and decoder (Telonics, USA). Each transmitter transmitted at a set frequency in the 150 mHz or 160 mHz band, the temperature being translated into a succession of beeps. Beeps were recorded from individual *M. domestica* (held in the colony room) at 5 minute intervals for up to 30 minutes every hour depending on the number of animals employed by the system at the time. The intervals between these beeps were then converted into temperature readings from the determined calibration curves. Recordings from individual *M.domestica* and *P. breviceps* in the temperature gradient were made every 6 seconds (and one-minute recordings downloaded) and 15 minute averages calculated. Diurnal variation patterns of core Tb were analysed using Fourier analysis and significance determined using Students' t-tests. Fourier analysis is regarded to be an accurate method for analysing complex waveforms which closely resemble actual circadian rhythms (Refinetti, 1993).

2.2c *Monodelphis domestica*

Basal circadian patterns of core Tb were determined from eighteen juvenile and adult *M. domestica* maintained at the colony temperature (ie., 28-30°C). Juvenile animals were males aged between 59 and 126 days old (sexually immature) at the time of implantation. A total of seven juvenile animals were analysed. Eleven adult male animals aged between 277 and 1118 days old at the time of implantation were utilised. Recordings of Tb were made 2 days post-implantation and continued for a minimum of eighteen days with data analysed only from those days where the recording apparatus allowed for continuous measurements to be recorded.

Tb rhythms in cold conditions were also investigated in adult animals. Four adult males were exposed to temperatures of 10°C, 20°C and 30°C and circadian rhythms of core Tb established. Animals were placed in

a temperature controlled cabinet placed in the same room as the colony and recordings were made from transmitter signals. Body mass was monitored regularly, particularly during exposures at colder temperatures and animals were removed from the cold temperature cabinet if consistent weight loss was noted.

Circadian Tb rhythms of five adult males were also analysed whilst selecting selected Tas in a longitudinal temperature gradient (refer to 2.3). The effects of bacterial endotoxin and hypoxia on selected Ta, core Tb and metabolic rates were also investigated (refer to 2.4 and 2.5).

2.2d *Petaurus breviceps*

Tb rhythms of one adult *P. breviceps* were analysed whilst in an aluminium longitudinal thermal gradient ranging from 15.72 to 40.01°C (refer to 2.3). The effects of bacterial endotoxin and hypoxia on selected Ta and core Tb were also assessed in this animal while in the thermal gradient (refer to 2.4 and 2.5).

2.3 Determination of Selected Ambient Temperatures

The initial body mass of each animal was recorded with an analytical balance (Selby Anax) to an accuracy of 1g. Activity and selected Ta were determined in unrestrained animals using a longitudinal temperature gradient in a room lighted by fluorescent lights. The gradient was borrowed from Dr Peter Frappell (LaTrobe University) and was constructed from 10mm thick aluminium and had interior dimensions of 1380mm (length) x 100mm (width) x 115mm (height). The top of the gradient was covered with a blackened out Plexiglas cover with a viewing area 10mm wide running along its centre. The Plexiglas was equipped with ports for three drinking bottles and incurrent and excurrent air. Water at a temperature of 50-55°C was circulated through one end of the gradient while 10°C water was circulated through the opposite end. Absorbent pellets covered the floor of the gradient and food and fresh drinking water were available ad libitum.

This arrangement produced an approximately linear thermal gradient with air temperatures ranging from 14.9°C to 40°C for *M. domestica* (15.7°C to 40°C for the *P. breviceps*). The thermal gradient was calibrated with air flowing through the system at approximately 1 L/min using a thermocouple and electronic recorder (Digi-Sense). The thermocouple and electronic recorder were calibrated against a certified mercury thermometer to a maximum error of +0.2°C. Introduction of an animal made no significant difference to the gradient measured. Opposing pairs of LEDs and photocells were evenly spaced at 36mm intervals along the tube for the detection of animal position. Animal position was then used to determine Ta selected. A radio antenna detected the Tb signal from the surgically implanted abdominal transmitter. Photocell and temperature decoder signals served as input to a MacLab system (AD Instruments) and Macintosh computer (SE or LC) to record data.

An open-flow respirometer system was used. Incoming air was dried using two tubes of indicating Drierite (anhydrous CaSO4) before entering the chamber. Excurrent air also passed through indicating Drierite prior to entering the oxygen sensor. Airflow through the chamber was controlled with a flowmeter. The flowmeter was calibrated under the same temperature and back pressure as in the experimental procedure. Air flow through the chamber ranged from 0.88L/min to 1.02L/min. Dry gas mixtures (compressed air or approximately 10% O2) were flowed continuously through the thermocline at a rate of approximately 1L/min. Air flow equilibrated throughout the chamber within 2-3 minutes. Direction of flow through the thermal gradient was related to temperature selection rather than orientation to the air supply. Oxygen concentration of excurrent gas from the gradient apparatus was measured by an Applied Electrochemistry S-3A Oxygen Analyser.

An animal was placed in the gradient and the Plexiglas lid sealed while dry air was circulated from the warm end towards the cool end at a rate of 1L/min. The position of the opossum or sugar glider was noted at 6

second intervals and core Tb measured. Core Tb was then averaged for each 15 minute period (X±S.D.). Selected Tas were determined using a standard curve of position in the gradient versus air temperature. Each animal was left undisturbed in the gradient for an initial 24-hour period followed by at least three consecutive days of recordings. Continuous measurements were made for up to 7 days.

Selected Tas and activity data were statistically analysed using Fourier analysis to determine any circadian patterns and paired t-tests were used to determine significance. Differences with probabilities less than 0.05 were accepted as significant.

2.4 The Effects of Fever and Hypoxia on Thermoregulation

Selected Tas and core Tb were analysed during fever, hypoxia and fever and hypoxia in six adult male *M. domestica* and one adult female *P. breviceps*. These measurements were conducted whilst the animal was in a longitudinal temperature gradient.

All febrile and hypoxic data were statistically compared using Students t-tests.

2.4a Determination of a Febrile Response to Bacterial Endotoxin

To induce a febrile response, bacterial endotoxin was injected into the animal. The endotoxin used was supplied by Sigma (L-2637 lipopolysaccharide from *Eschericia coli* serotype 055:B5) with a concentration of 1mg/ml. The experimental animals received doses of 1mg/kg. The injections were given intramuscularly into the hindleg between 09:30h and 10:00h. Physiological saline (0.9%NaCl) was used as a control and was administered at the same volume as the endotoxin and at the same times of the day.

A self-pairing experimental system was used for five male *M. domestica* and one female *P. breviceps*. Animals were injected with

physiological saline and Tb and selected Ta monitored continuously for 24 hours. They were then injected with endotoxin and similarly monitored for a further 24 hours. Monitoring involved recording core Tb and the position of the animal in the gradient at six second intervals. Fifteen minute averages for Tb were then determined and the selected Ta every six seconds calculated by a standard curve of position versus Ta. An additional control experiment was also performed on two animals whereby physiological saline was administered instead of endotoxin in the second injection and Tb and activity were monitored for 24 hours.

2.4b Determination of Hypoxic Effects on Thermoregulation

While in the temperature gradient each animal was exposed to normoxic conditions (21% O_2) for 24 hours followed by a 24 hour exposure to hypoxic conditions (10-15% O_2). Hypoxic conditions were created using a mixture of compressed air and nitrogen to achieve the desired O_2 level. A varied O_2 level was used for each animal as the gas mixture did not always ideally approximate 10% O_2 despite efforts to maintain a fairly consistent level. Oxygen concentration of excurrent gas from the gradient apparatus was measured by an Applied Electrochemistry S-3A Oxygen Analyser. Selected Tas and core Tbs were simultaneously determined under each condition (ie normoxia and hypoxia) at six second intervals. A standard curve of position in gradient versus air temperature was used to determine selected Ta each six seconds and 15 minute averages of core Tb were calculated.

2.4c Determination of the Effects of a Combination of Bacterial Endotoxin and Hypoxia on Thermoregulation

In addition the effects of hypoxia and fever simultaneously on selected Ta and core Tb were investigated. Animals were injected intramuscularly with endotoxin and after two hours hypoxic conditions were induced for a period of up to six hours while the animal was in a longitudinal temperature gradient. During this time selected Ta and core Tb were simultaneously measured every six seconds. As for previous

experiments on the effects of hypoxia and the effects of endotoxin, 15 minute averages of core Tb were determined and a standard curve was used to calculate selected Ta.

2.5 Determination of Metabolic Rate and the Effects of Hypoxia

Normal circadian patterns of metabolic rate were recorded for six adult *M. domestica*. Animals were fasted for 3 hours and then placed in a metabolic chamber in a constant Ta (30°C) and metabolic rate measured. Rates of oxygen consumption (VO_2) were measured at Tas around 10°C, 15°C, 20°C, 25°C and 30°C using an open-airflow system with a S-3A oxygen analyser (Applied Electrochemistry, Pa, U.S.A.), a flow controller (Applied Electrochemistry Model R-1) and a zirconium oxygen sensor (Applied Electrochemistry, Model N-37M).

Measurements of $\%O_2$ in the outlet air from the chamber were recorded simultaneously over a 24 hour period at a Ta of 30°C. VO_2 was calculated from equation 4a of Withers (1977) and corrected to standard conditions (STP:O°C, 760 mmHg, dry air). Estimations of Basal Metabolic Rate (BMR) were then determined from 24-hour recordings.

The effect of hypoxia on basal and resting metabolism was then assessed in six *M. domestica* using a metabolic chamber (volume; 2.2L). Air flow through the chamber was controlled by a flowmeter calibrated under the same temperatures and back pressure as the experimental procedure. Flow rate was 0.3-0.4L/min. Each animal was starved for 3 hours and placed in the metabolic chamber at Tas of 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C for 30-60 minutes under normoxic conditions. Twenty four hours later the experiment was repeated under hypoxic conditions (12-15% O_2 levels). The hypoxia mixture was produced using a combination of compressed air and nitrogen to achieve the desirable O_2 level. The TNZ was determined for each individual animal from the recordings of VO_2. In the event that this zone was not apparent, metabolic rate was estimated from the highest Ta (ie., 30°C) which had previously fallen within the TNZ of the

species. As the animals were in a fasted state throughout the recording period, the VO_2 measurement is a relatively reliable indication of basal metabolic rate. Data were statistically analysed using Students t-tests and ANOVA.

2.6 Thyroid Hormone Assays

Plasma levels of thyroxine (T_4) and triiodothyronine (T_3) were determined from *M. domestica* at normal conditions and during exposure to cold temperatures. Blood was obtained via cardiac puncture (under fluorothane anaesthesia), centrifuged, and plasma retained and stored at -80°C.

2.6a Determination of Triiodothyronine Levels

Total plasma T_3 levels were determined using an Amerlax-M RIA (radioimmunoassay) kit IM.3001/IM.3004 (Kodak Clinical Diagnostics). Plasma from experimental animals (50µl) and standards (50µl) ranging from 0.5 nmol/l to 12.0 nmol/l were incubated with 500µl [^{125}I]T_3 reagent and 500µl Amerlex-M T_3 antibody reagent for 60 minutes at 37°C. Non-specific binding was determined in tubes set up with 50µl standard serum incubated with 500µl [^{125}I]T_3 reagent and 500µl Amerlex-M NSB reagent. After incubation the tubes were attached onto an Amerlex-M Separator base for 15 minutes. After separation the supernatant liquids were poured and discarded and the tubes were drained on a pad of absorbent paper for 5 minutes. The rims of the inverted tubes were then firmly blotted to remove adhering droplets before tubes were returned to the upright position. All tubes were counted in a gamma counter (Multigamma).

2.6b Determination of Thyroxine Levels

Total plasma T_4 levels were determined using an Amerlax-M T_4 RIA kit IM.3011/IM.3014 (Kodak Clinical Diagnostics). Plasma from

experimental animals (50µl) and standards (50µl) ranging from 0 nmol/L to 320 nmol/L were incubated with 500µl [^{125}I]thyroxine solution and 500µl Amerlex-M T_4 antibody reagent for 45 minutes at room temperature (18-28°C). Non-specific binding was determined in tubes set up with 50µl standard serum incubated with 500µl [^{125}I]T_3 reagent and 500µl Amerlex-M NSB reagent. After incubation the tubes were attached onto an Amerlex-M Separator base for 15 minutes. After separation the supernatant liquids were poured and discarded and the tubes were drained on a pad of absorbent paper for 5 minutes. The rims of the inverted tubes were then firmly blotted to remove adhering droplets before tubes were returned to the upright position. All tubes were counted in a gamma counter (Multigamma).

2.7 Analytical and Statistical Analysis

Mean±SD at 15 minute intervals throughout 24 hour cycles were determined for all Tbs recorded. Significance of daily Tb between juvenile and adult *M. domestica* and between adult *M. domestica* in a constant temperature room and adult *M. domestica* in a longitudinal temperature gradient were determined using Students two-sample t-tests and single factor ANOVA. Significant differences between Tb recorded at Tas of 30°C, 20°C, 15°C and 10°C were determined for four adult *M. domestica* using ANOVA and t-tests. Daily mean±SD was also determined for selected Ta under control conditions. Significant differences between thyroid hormone levels in adult and juvenile *M. domestica* were determined using single factor ANOVA.

As Tb (and possibly selected Ta) follows a circadian cycle (see Chapter 3), a complete analysis of how experimental or other effects alter Tb needs to include not only mean Tb, and variability, but also, where appropriate, some measure of periodicity. Accordingly, the following

descriptive measures have been used: Mean±SD, minimum, maximum, range, period, and acrophase (timing of peak).

Fourier analysis (STATISTICA 4.1; Statsoft, Oklahoma) was used to determine circadian patterns (ie period) of recorded Tb and selected Ta under normal, hypoxic, febrile and febrile+hypoxic conditions. This form of analysis has been shown to be a superior for of analysis for complex waveforms such as that observed in actual circadian rhythms (Refinetti, 1993). Significant effects of endotoxin and hypoxia on core Tb and selected Ta were also determined from Two-factor ANOVA and two-sample paired t-test analysis. Degrees of freedom were determined from (n-1). 95% confidence intervals were used ($p<0.05$).

CHAPTER 3

CIRCADIAN RHYTHMS OF CORE BODY TEMPERATURE

3.1 Introduction

The circadian control of core Tb is a complex interaction between the thermoregulatory system and a number of other physiological systems in the body (Fuller and Sulzman, 1982). Rhythmical variations of core Tb exist in many mammals (eg Bligh and Harthoorn, 1965) and may or may not be due to a shift in the thermal set-point (believed to exist in the hypothalamus). Daily Tb rhythms are endogenously generated and usually follow a 24 hour period (Aschoff, 1970). These rhythms may be entrained on environmental cues or on levels of activity. Circadian rhythms of hypothalamic and colonic temperatures are similar in the squirrel monkey yet mean temperatures of these rhythms are controlled differentially (Fuller, 1984). Mammalian Tb rhythms typically represent a monophasic shape (eg Hoban et al., 1985; Honma and Hiroshige, 1978) although a biphasic shape with exaggerated amplitude has been observed in the wombat, *Vombatus ursinus* (Peters and Rose, 1979), the domestic cat, *Felis catus* (Randall et al., 1987) and the tree shrew, *Tupaia belangeri* (Refinetti and Menaker, 1992a).

Many studies on daily Tb fluctuations have focussed primarily on adult animals with the presence of rhythmical variations in Tb in young or juvenile animals often ignored. Nuesslein-Hildesheim et al., (1995) observed juvenile circadian core Tb rhythms in rats yet found no such rhythms to exist in juvenile rabbits. At a similar developmental stage, rabbit pups maintain high levels of metabolism and core Tb throughout the day yet rat pups show clear circadian rhythms with significant decreases in Tb and metabolic rate during the morning hours (Nuesslein-Hildesheim et al., 1995). Circadian rhythms of body temperature have also been observed during the first week of life in rat pups (Nuesslein and Schmidt, 1990;

Mumm et al., 1990) and once independent from their mother (Kittrell and Satinoff, 1986). However circadian patterns of other juvenile mammals have not been observed. It is highly unlikely that only juvenile rats would be capable of maintaining cyclical variations in Tb given the similarities known between mammals in Tb regulation and development. Further research on juvenile Tb rhythms in a diverse range of mammalian species is therefore needed.

Circadian patterns of Tb have been determined in many marsupial species (Morrison, 1965) yet differences between adults, juveniles and newborns have not been highlighted. The development of thermoregulation in marsupials is unique as it occurs within the pouch environment in most species (Hulbert, 1988). The attainment of full thermoregulatory mechanisms occurs along different time scales in different species (Morrison and Petajan, 1962; Shield, 1966; Setchell, 1974; Gemmell and Johnston, 1985; Gemmell et al., 1987; Gemmell and Cepon, 1993; Holloway and Geiser, 2000) but usually occurs by the time of weaning (Hulbert, 1988). Mean Tbs of juvenile (post-weaned young) and adult marsupials do not differ significantly (Douglas, 1990) yet some differences may exist throughout the circadian phases and patterns. Such possible differences have not yet been investigated in marsupial species.

An allometric relationship has been shown to exist between body size and the maximum amplitude of the circadian increase in Tb between a non-primate mammal's daily rest phase and daily activity phase (Aschoff, 1982). Aschoff (1982) suggests that the maximum Tb during the activity phase is fairly independent of body size yet the minimum Tb during the rest phase increases with increasing body size. As a result the range of Tbs exhibited decreases with increasing body size (Aschoff, 1982). This is apparent when comparing different animals or different species (eg Lovegrove and Heldmaier, 1994). It would seem feasible to assume therefore that younger animals would exhibit a greater range of Tbs within a daily rhythm than older animals of the same species. Indeed, according to Refinetti and

Menaker, (1992b) interspecies variations and intraspecies modulations in daily Tb control appear related to gender, age, stage of development and physiological state. Circadian rhythms observed in adult animals of a species may not be similarly observed in newborn or even juvenile animals of the same species. This is because thermoregulation has not yet fully developed in the younger animals.

Circadian rhythms of Tb in both adult and juvenile *M. domestica* were investigated and compared in this study. It was hypothesised that Tb rhythms would exist over a 24-hour cycle in adult marsupials but not in juvenile animals (as thermoregulation may not yet be fully developed in these latter animals). If such rhythms occurred it was also proposed that they would be uni-modal rather than bi-modal.

3.2 Results

Transmitters were successfully implanted into seventeen out of eighteen adult and juvenile male *M. domestica*. One juvenile animal (MOJ5) was unable to be used for experimental recordings due to the irritation of the stitched abdominal region by the animal two days after the operation. Another animal, an adult (MOAG14) had a transmitter successfully implanted however recordings were not viable and a battery problem rendered this animal unsuitable for analysis. Recordings of core Tb were therefore collected from a total of sixteen adult and juvenile *M. domestica*.

3.2a Measurements of Core Body Temperatures

i) Juvenile *M. domestica*

Circadian patterns of Tb were recorded from six juvenile male *M. domestica*. Continuous measurements of Tb obtained from each juvenile animal are shown in Appendix 3. Three of the six animals analysed (MOJ7,

MOJ8 and MOJ9) were returned to their mother and siblings once the radiotransmitter was implanted and recordings were then made.

Tb was recorded from juvenile *M. domestica* at 28-30°C (the temperature of the colony holding facility) with significant differences observed between recordings from individual animals ($F=8237$: $p<0.05$). Daily means of core Tb were determined from the continuous recordings and are presented in Table 3.2.1. Average daily core Tb of individual animals ranged from 31.8±0.6°C to 34.8±0.5°C with a mean of 33.2±1.0 °C (see Table 3.2.1). Mean daily Tbs recorded from juveniles in the presence of their mother and siblings (ie MOJ7, MOJ8 and MOJ9) were less variant than juvenile animals placed in individual cages (with variances of 0.3 and 2.3 respectively), however mean Tbs were not significantly different to juveniles in individual cages ($t=0.50$; $p>0.05$).

Table 3.2.1 Summary of daily core body temperatures of juvenile *Monodelphis domestica* as determined from continuous recordings over successive 24 hour periods.

Animal	No. of days analysed	Daily Tb (°C) (X±SD)	Minimum Tb (°C)	Maximum Tb (°C)	Tb Range (°C)
MOJ2	10	31.8±0.6	30.9	33.3	2.4
MOJ3	10	34.8±0.5	34.0	35.9	2.0
MOJ4	15	33.7±0.7	32.5	35.3	2.8
MOJ7	3	33.3±0.3	32.8	34.1	1.4
MOJ8	29	32.4±0.3	31.7	33.0	1.3
MOJ9	28	33.2±0.3	32.6	34.0	1.3

Tbs were recorded every five minutes from juvenile *M. domestica* and continuous measurements of Tb observed in each animal are shown in Appendix 3. Figures 3.2.1a and 3.2.1b show a typical recording of continuous Tb from a juvenile *M. domestica*. 15 minute means±SD were determined from a number of experimental days for each animal. A mean minimum Tb of 32.7°C occurred at 0900 hours and a mean maximum Tb of

33.9°C was exhibited at 2115 hours in juvenile animals. Peak Tbs were observed during nocturnal hours in all juvenile animals. Day Tbs were significantly lower than night Tbs in all individual animals as determined by a paired t-test ($t=4.4$; $p < 0.05$).

Figure 3.2.1a Typical Continuous Body Temperature (Tb) Recording from a Juvenile *M. domestica* (MOJ9) in a cage with its mother and siblings
[Three days of recording shown]

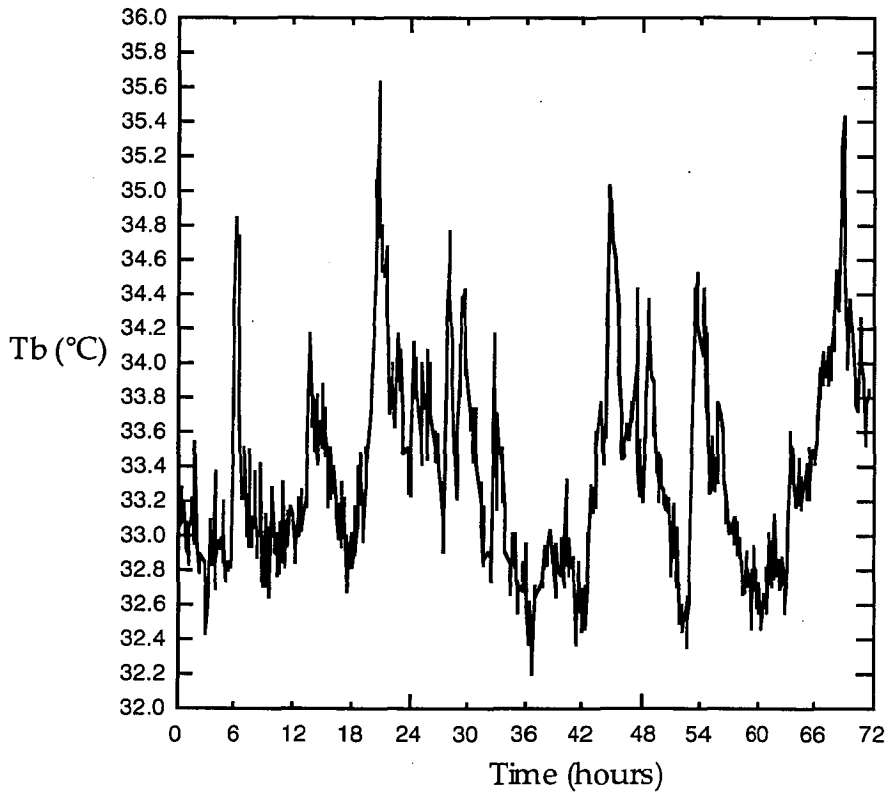
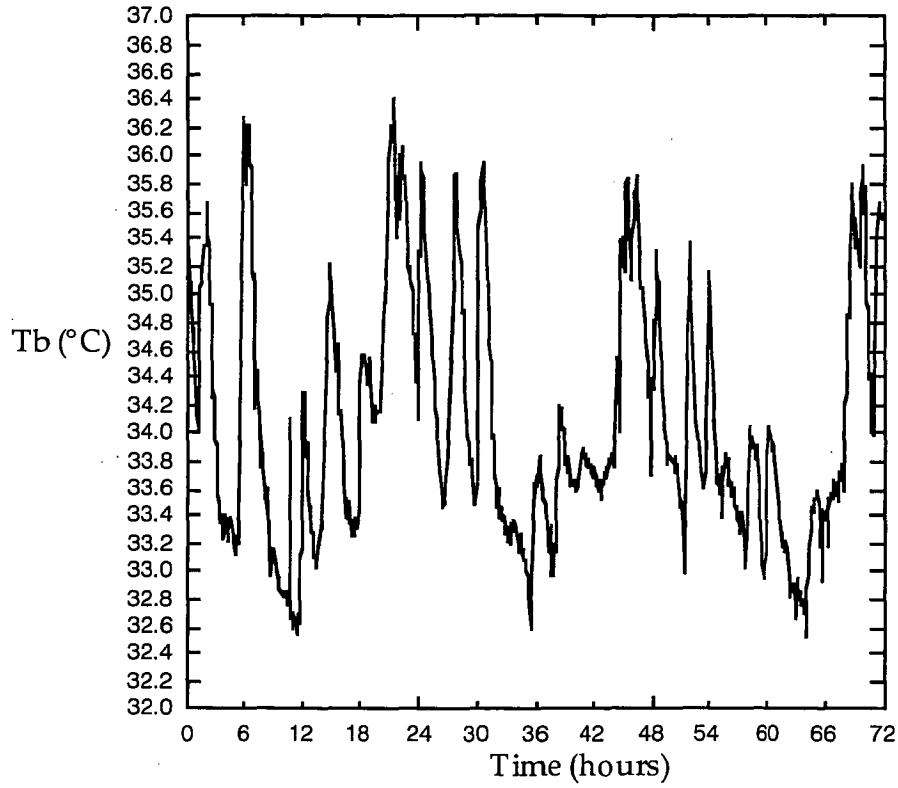


Figure 3.2.1b Typical Continuous Body Temperature (Tb) Recording of a Juvenile M. domestica (MOJ4) while alone in a cage
[Three days of recording shown]



Fourier analysis (Statsoft STATISTICA) of five minute Tb recordings for each animal indicated the lack of a distinct circadian rhythm in three of the animals although a 24-hour rhythm was evident in MOJ3, MOJ4 and MOJ7 (see Appendix 9 for analysis plots of each animal). Data were analysed using a single series Fourier analysis and results plotted by period using transformations in which the mean was subtracted and detrended. The periods are given for each analysis in Appendix 9. Figures 3.2.2a and 3.2.2b show a typical Fourier analysis plot of Tb for a juvenile *M. domestica*. Table 3.2.2 shows the amplitude of Tb, acrophase, and period for each juvenile animal.

Figure 3.2.2a Typical Single Series Fourier (Spectral) Analysis of Body Temperature in a Juvenile Opossum (MOJ4) alone in a cage maintained at 20-30°C

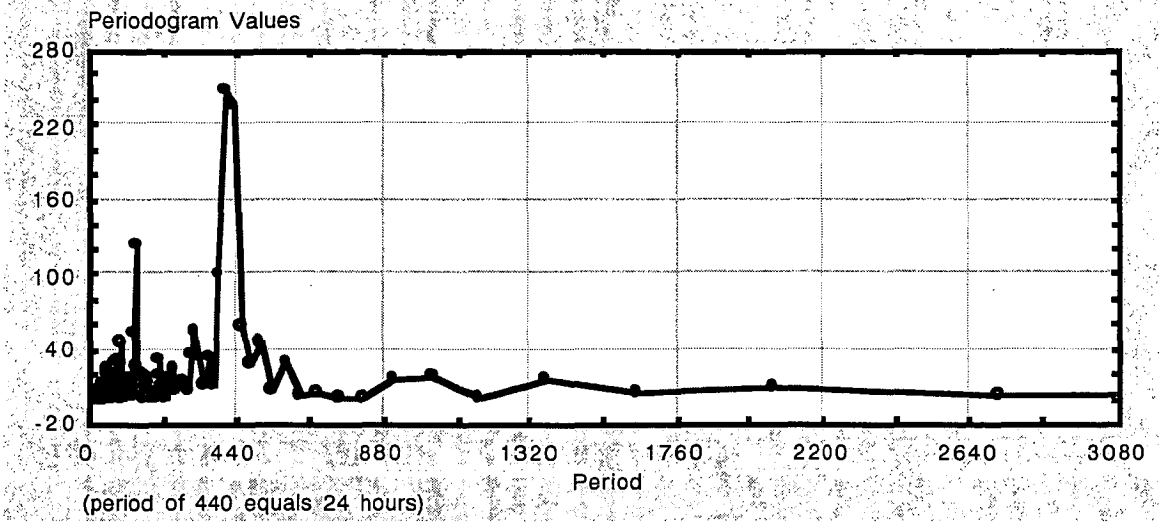


Figure 3.2.2b Typical Single Series Fourier (SPectral) Analysis of Body Temperature in a Juvenile Opossum (MOJ9) while in a cage with its mother and siblings in a 28-30°C room

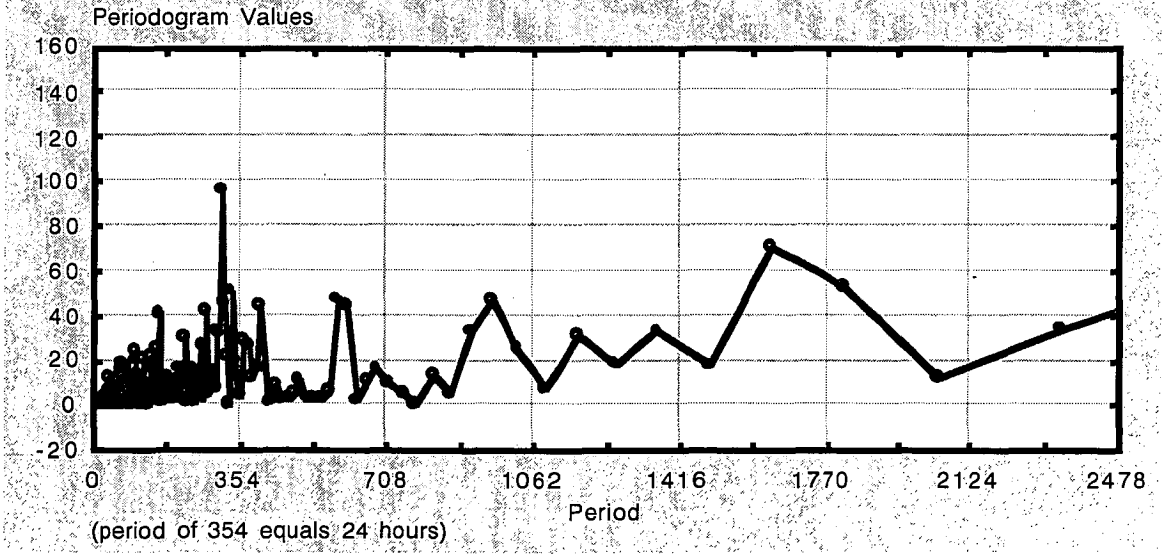


Table 3.2.2 Analysis of Body Temperature in each Juvenile Animal: Amplitude, acrophase (timing of peak), and period
[period was determined from Fourier analysis; amplitude is the difference between the maximum and minimum Tb; acrophase is shown as 24-hour clock time]

Animal	Amplitude (°C)	Acrophase	Period (hours)
MOJ2	6.2	13:41	40.0
MOJ3	3.4	01:22	24.0
MOJ4	5.5	06:23	24.0; 26.4
MOJ7	2.1	04:01	24.0*
MOJ8	5.7	02:06	21.5
MOJ9	4.9	20:52	20.8

*MOJ7 results were analysed from only two days of recordings

ii) Adult *M.domestica*

Tb rhythms of five adult *M. domestica* in the colony holding facility (colony adults) were measured. In addition, core Tb rhythms were determined from five adult *M. domestica* in a temperature gradient (gradient adults). The colony holding facility was set at a Ta of 28-30°C and the temperature gradient ranged from 15-42°C. The latter set-up allowed the animals to behaviourally select a preferred Ta. As for juvenile animals, Tbs from the initial two days of recording were not included in the analysis to allow time to recuperate from surgical procedures. Adult animals were of reproductive age (ie, at least six months of age). Adult animals in the colony holding facility were aged between 230 and 859 days old at the time of implantation and recordings continued for a minimum of eleven days. Adult animals used in the temperature gradient were aged between 474 and 1318 days old at the time of implantation and recordings continued for a minimum of five days.

Significant differences between Tb recordings from individual adult animals do exist ($F=3792$; $p<0.05$). Average daily core Tbs for each adult animal are presented in Table 3.2.3. The average daily Tb of colony adults was between 33.5±0.5°C and 34.9±0.7°C. The average daily Tb of gradient adults was between 34.3±0.6°C and 34.8±0.6°C. Gradient adults exhibited Tbs with a variance of 0.04 which was much lower than the variance between mean Tbs in colony animals (ie. 0.3). Mean daily Tbs were not significantly different between gradient and colony animals ($t=-0.6$; $p>0.05$).

Table 3.2.3 Summary of Daily Core Body Temperatures (Tb) of Adult *Monodelphis domestica* as determined from continuous recordings over successive 24 hour periods.

Animal	Colony or Gradient	Tb (°C) (Mean±SD)	Minimum Tb (°C)	Maximum Tb (°C)	Tb Range (°C)
MOAC1	colony	34.4±0.6	33.4	35.8	2.4
MOAC6	colony	34.2±0. 5	33.3	35.3	2.0
MOAC10	colony	33.52±0.5	32.7	34.4	1.7
MOAC11	colony	34.6±0.7	33.8	35.4	1.6
MOAC12	colony	34.9±0.7	34.0	36.0	2.0
MOAG13	gradient	34.5±0.5	33.6	35.6	1.9
MOAG15	gradient	34.3±0.6	33.4	35.4	1.9
MOAG16	gradient	34.6±0.4	33.9	35.3	1.4
MOAG17	gradient	34.3±0. 6	33.6	35.5	1.9
MOAG18	gradient	34.8±0.6	33.5	36.0	2.5

Tb was recorded from colony animals (MOAC) at five minute intervals and from gradient animals (MOAG) at six second intervals. All data were pooled into 15 minute means. As for juvenile animals, means±SD at 15 minute intervals were then determined from a number of experimental days for each animal. The minimum number of days analysed in colony adults was six days. Gradient adults were analysed for a period of at least three days.

A typical recording of continuous Tb measurement from an adult male *M. domestica* in a constant temperature room is shown in Figure 3.2.3a. Figure 3.2.3b illustrates a typical recording of an adult male *M. domestica* in a

longitudinal thermal gradient. Continuous measurements of Tb observed in individual adult *M. domestica* are illustrated in Appendix 2. Acrophase of Tb (ie time of maximum Tb) and maximum and minimum Tb during a circadian cycle differed between adults at colony Ta and adults at gradient Ta. Colony adults exhibited a mean minimum core Tb of 33.7°C at 0830 hours and a mean maximum core Tb of 35.2°C at 2215-2230 hours. In comparison, gradient adults exhibited a mean minimum of 33.8°C at 1415 hours and a mean maximum of 35.4°C at 0445 hours. When considering the adults as a total group, the minimum Tb is 33.82°C at 0830 hours and the maximum is 35°C at 2230 hours. Temperatures recorded during nocturnal hours were significantly higher than those during daylight hours in all adult animals ($t=4.4$; $p<0.05$).

Figure 3.2.3a A Typical Continuous Tb Recording of an adult *M. domestica* (MOAC10) in a constant condition room (28-30°C)
[Three days of recording shown]

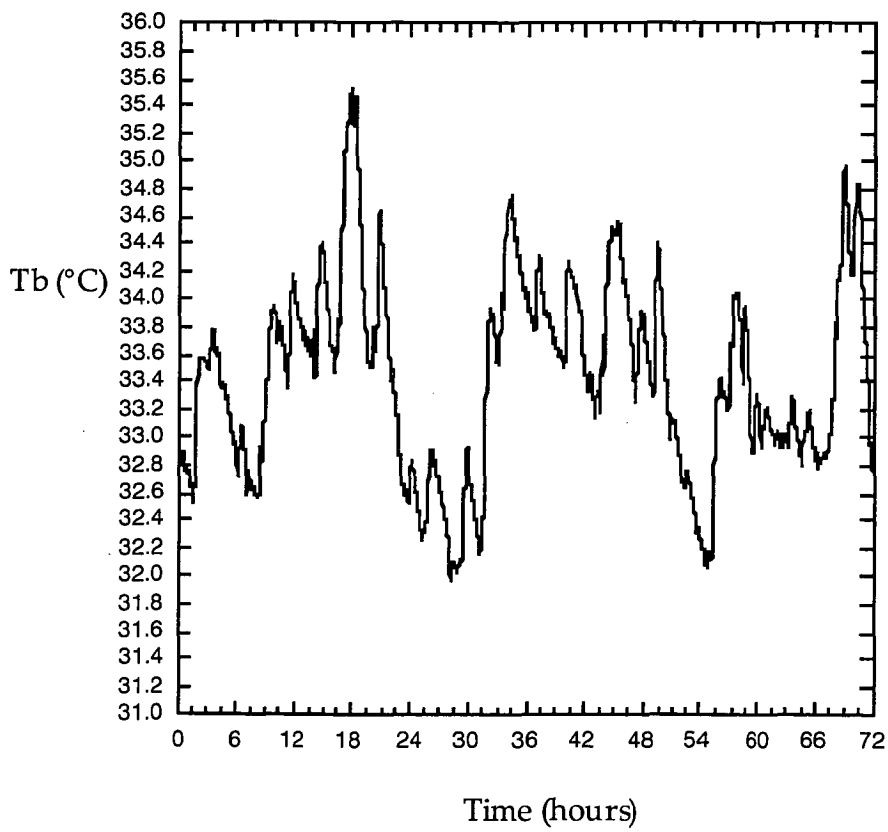
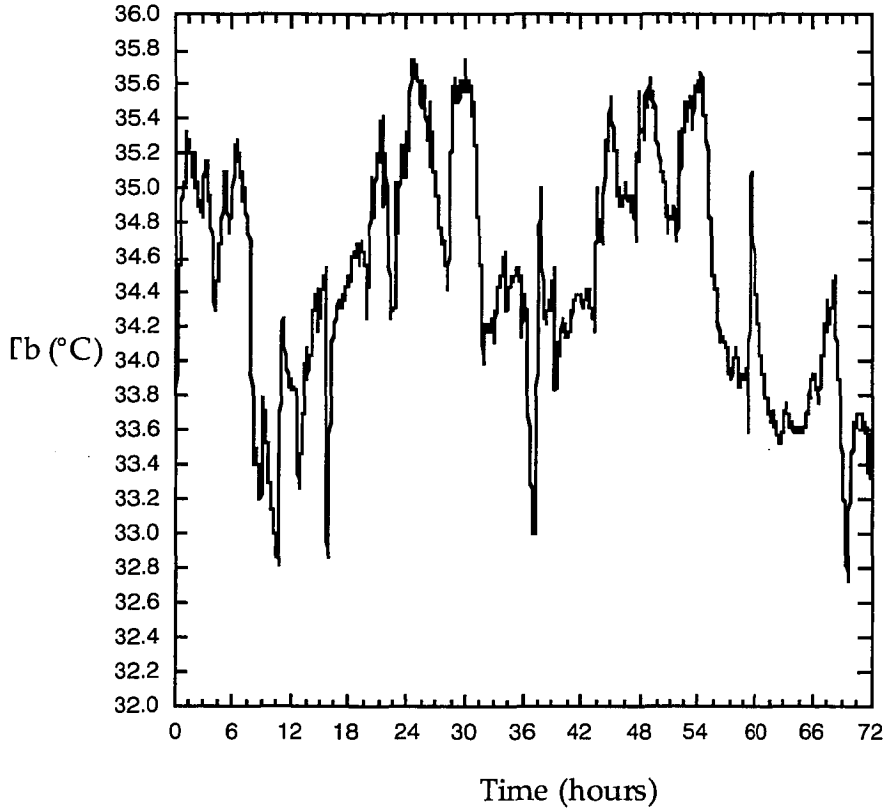


Figure 3.2.3b A Typical Continuous Body Temperature (Tb) Recording from an adult *M. domestica* (MOAG13) in a longitudinal thermal gradient
[Three days of recording shown]



Fourier analysis of five minute (for MOAC) and six second (for MOAG) Tb recordings indicated the presence of a circadian rhythm in each animal. Data were analysed using a single series Fourier analysis and results plotted by period using transformations in which the mean was subtracted and detrended (see Appendix 9). Table 3.2.4 shows the amplitude of Tb, acrophase, and period for each adult animal. The periods are given for each analysis in Appendix 9. A typical Fourier analysis of Tb measurements from an adult male *M. domestica* in the colony (ie MOAC10) and an adult male *M. domestica* in a thermal gradient (ie MOAG13) are shown in Figures 3.2.4a and 3.2.4b respectively. Circadian rhythms were observed to be more pronounced in animals in a thermal gradient than those maintained at a relatively constant room temperature.

Figure 3.2.4a Typical Single Series Fourier (Spectral) Analysis of Body Temperature from an Adult Opossum (MOC10) maintained in a room at 28-30°C

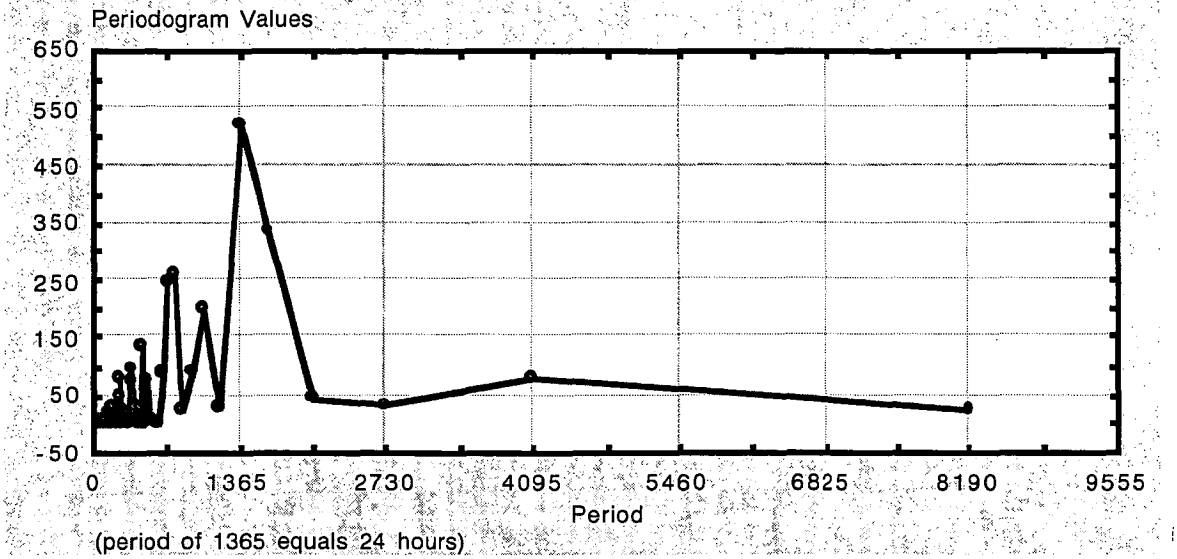


Figure 3.2.4b Typical Single Series Fourier (Spectral) Analysis of Body Temperature from an Adult Opossum (MOAG13) maintained in a longitudinal thermal gradient

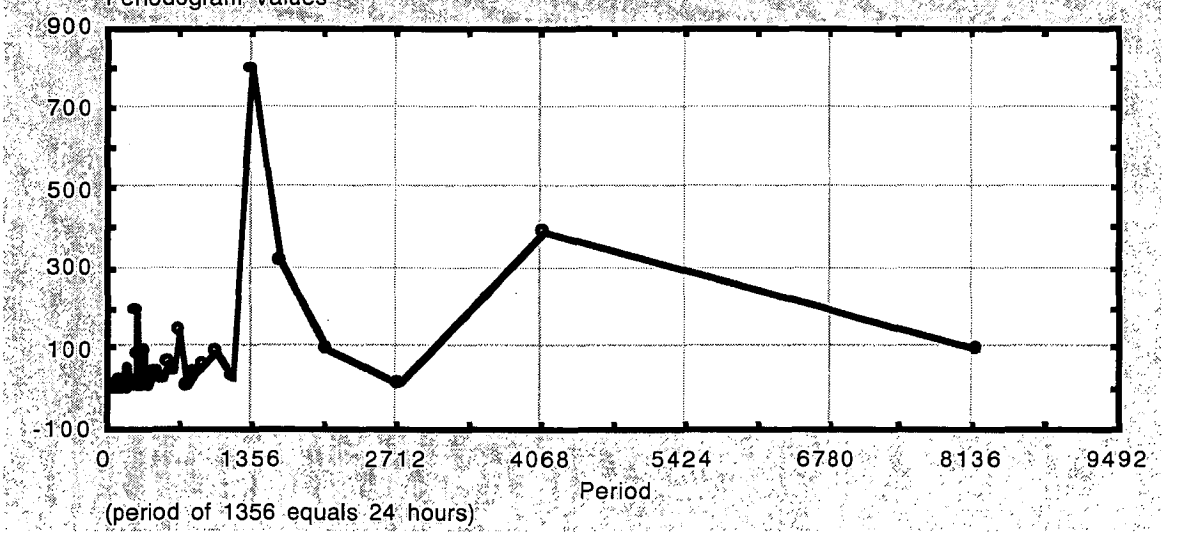


Table 3.2.4 Analysis of Body Temperature in each Adult *M. domestica*: Amplitude, acrophase (timing of peak), and period
[period was determined from Fourier Analysis; amplitude is the difference between the minimum and maximum Tb; acrophase is shown as 24-hour clock time]

Animal	Amplitude (°C)	Acrophase	Period (hours)
MOAC1	6.5	22:46	24
MOAC6	3.2	13:08	24
MOAC10	4.6	07:11	24
MOAC11	4.1	19:54	24
MOAC12	4.0	21:48	24
MOAG13	4.1	00:33; 00:45; 0:51; 06:07	24
MOAG15	2.5	04:04; 04:47	24
MOAG16	2.6	02:59	24
MOAG17	3.0	00:20	24
MOAG18	3.9	04:41	24

iii) Adult and Juvenile *M. domestica*

Mean daily Tb in adults and juveniles differed. Table 3.2.5 shows the daily Tbs determined for each group of *M. domestica*. Juvenile animals generally exhibited a lower mean daily temperature than adults although core Tbs were not significantly different between the two groups ($t=1.9$; $p<0.05$). Tbs recorded from adults in a temperature gradient were less variant than those in the colony holding room. However, mean Tbs of colony adults and gradient adults were not significantly different ($t=0.48$; $p<0.05$).

Table 3.2.5 Daily mean core body temperatures (Tb) in adult and juvenile *Monodelphis domestica* as determined from continuous recordings over successive 24 hour periods.

Group	Number in group	Tb (°C) (X±SD)	Minimum Tb (°C)	Maximum Tb (°C)	Tb Range (°C)	Variance (°C)
Juveniles	6	33.2±1.04	32.4	34.8	2.4	1.1
Adults	10	34.4±0.38	33.5	34.9	1.4	0.3
Colony adults	5	34.3±0.51	33.5	34.9	1.4	0.3
Gradient Adults	5	34.5±0.2	34.3	34.8	0.5	0.04
All	16	34.0±0.82	31.8	34.9	3.1	0.7

Circadian patterns of Tb were evident in all adult but not in all juvenile *M. domestica* with higher temperatures generally observed during the evening hours in both adult and juvenile animals. The range of Tb observed in a 24 hour period in juveniles is 1.2°C compared with 1.5°C in adults (1.5°C in colony adults and 1.6°C in gradient adults). Combining adult and juvenile temperatures produces a range of 1.1°C throughout a 24 hour period with a mean minimum Tb of 33.4°C at 0830 hours and a mean maximum Tb of 34.5°C at 2115 hours. Variance of Tb for all animals was also increased in comparison to adult groups due to the effects of the varying juvenile Tbs as shown in Table 3.2.5.

3.3 Discussion

3.3.1 Mean Daily Core Body Temperatures

Body temperatures recorded in this study were comparable to those previously reported in these species yet are somewhat lower than the average value of 35.5°C reported for marsupials in general (Dawson and Hulbert, 1970). A mean Tb of 32.6±0.4°C for male and female *M. domestica* (Dawson and Olson, 1988) is comparable to the juvenile Tb recorded in the current study (ie, 33.2±1.04°C). However, it is a lower finding than the average daily temperature of 34.4±0.38°C recorded for adults in this study.

These differences are probably attributable to the methodology used to measure the core Tb (ie rectal thermocouple versus implanted temperature-sensitive transmitter) but cannot be conclusive as only one animal was analysed in this study.

Dawson and Olson (1988) measured the rectal temperature of warm-acclimated and cold-acclimated *M. domestica* before and after experimental protocols. The recordings represent the Tb at that particular time of day (ie. afternoon) at which the measurement was taken. Given the circadian changes in Tb observed in mammalian species, this does not represent a daily average core Tb. Tbs recorded in *M. domestica* during afternoon hours are lower than those measured during evening and early morning times. This supports the findings by Dawson and Olson (1988) for *M. domestica*.

This study has demonstrated the importance of measuring Tb over a 24 hour period in thermoregulatory studies. Using implanted temperature-sensitive radio-transmitters to record core temperature is a more reliable indicator of true core body temperature than rectal temperatures measured at timely intervals as circadian variations are not taken into consideration by the latter method. Comparing individual measurements of Tb over a 24-hour period indicates that recordings similar to those previously reported from rectal measurements do exist at various circadian time periods in *M. domestica*. However, as shown in this study, within a 24-hour period Tb can vary by more than 2°C.

There are significant differences in core Tb between some individuals of the same species due to circadian and other effects such as sleep. This was demonstrated in *M. domestica* with recordings of average Tbs ranging from 31.8°C to 34.9°C. Different animals of the same species have slightly different "set-points" for the hypothalamic thermoregulatory system which is presumably due to differences in environmental and genetic influences. This is supported by observations seen in previous mammalian studies of variations in daily Tb control (Refinetti and Menaker, 1992b). It is unlikely

that thermal behaviour is a key influence on Tb status as recordings of Tb in a thermal gradient tend to be similar to Tb recordings while in a constant temperature room although Tb is less variant when a range of Ta are available to the animal. The significance of thermoregulatory behaviour in *M. domestica* is discussed in Chapter 6 of this thesis.

When comparing juvenile and adult *M.domestica,* no significant differences between mean daily Tb was observed which is consistent with previous findings in other marsupial species (Douglas, 1990). The range of core body temperatures adopted by animals throughout a 24 hour phase also did not differ significantly between adult and juvenile animals. Thus, core Tb range does not necessarily decrease with increasing body size as proposed by Aschoff (1982) at least when comparing adults and juveniles of the same species. This was also shown by Douglas (1990) in both macropodid and non-macropodid marsupials. Despite a lack of significant difference between juvenile and adult Tb though, there is greater variance between measurements from younger animals. This may be due to some underdeveloped thermoregulatory mechanisms in juvenile animals, particularly with respect to NST and hypothalamic thermoregulatory control. As most of the Tb measurements were done at a relatively constant (and high) Ta, this may also explain the lack of variability between animals of different body size.

An interesting observation was the similarity between the range of Tbs measured from adults held at the colony temperature and those in a thermal gradient. Although the latter had the opportunity to selectively alter their core temperature through behavioural means, core temperatures were not significantly different between the two groups. However, variance of mean Tb in gradient animals was quite low in comparison to the colony animals indicating a possible role for behavioural thermoregulation. In fact, Tb results from animals in the gradient indicated the least variance while juvenile animals exhibited higher variance between individual animals. These results indicate that marsupials, like most endotherms, control their

Tb through physiological means however this control may be refined by behavioural selections of Ta. Opportunities to select preferred Ta would reduce the level of physiological adjustment required homeostatically to maintain thermogenesis. Behavioural thermoregulation will be addressed in more detail in Chapter 6 of this thesis.

3.3.2 Circadian Rhythms of Core Body Temperature

Circadian rhythms of Tb were found to be quite variable over a 24 hour period in *M. domestica*, however a distinct 24-hour rhythm was apparent in all adult animals. This is consistent with previous observations of continuous Tb recordings in marsupial species. Daily Tb has been found to be variable in *P. breviceps* (Dawson and May, 1984) and in the Tasmanian devil, *S. harrisii* (Guiler and Heddle, 1974) although rhythmical patterns are apparent. The Tasmanian bettong, *Bettongia gainardi* has been shown to have a typical nocturnal Tb rhythm with Tbs varying in a periodicity close to 24 hours (Rose et al., 1990) and the American opossum, *Didelphis virginiana* exhibits a prominent 24 hour rhythm comparable to that seen in eutherian mammals, despite obvious variances between Tb throughout 24 hours (Treagust et al., 1980). *M. domestica* exhibited a typical 24-hour Tb rhythm of a nocturnal mammal.

Juvenile *M. domestica* (both lactating and weaned) may or may not have a distinct 24 hour rhythm with great variance between Tbs recorded over a 24 hour period. Pronounced juvenile circadian core Tb rhythms distinctly different to adults have been observed in rats but not rabbits (Nuesslein-Hildesheim et al., 1995). This difference may be attributed to differences in maternal behaviour relating to thermoregulation in rodents and rabbits (Hudson and Distel, 1989; Schmidt et al., 1986, 1987). Rat pups isolated from maternal circadian influences at 4 days of age have been shown to exhibit circadian core Tb phases in phase with the light cycle (Nuesslein and Schmidt, 1990). As marsupials are born at a stage of immaturity which includes a poorly developed thermoregulatory system it

would be expected that juvenile Tb rhythms atypical of adults would not be observed.

Differences between circadian patterns of Tb in juvenile and adult *M. domestica* strongly indicates that the younger animals lack the finely tuned thermoregulatory system which is evident in the adult animals. All juvenile animals used in this study were yet to reach sexual maturity with the youngest animals still in the nest with their mother and siblings. These younger animals displayed less variant Tb recordings compared to older juveniles and would have used behavioural means of thermoregulation such as huddling to maintain core Tb. Interestingly, one animal (MOJ7) did show a 24-hour rhythm of Tb while in the nest with its mothers and siblings. This rhythm may be a reflection of the mother's rhythm of Tb or may indicate that this animal has developed its own circadian rhythm of Tb prior to leaving its mother at weaning. It would be of interest to determine to what degree this animal's rhythm was influenced by huddling as huddling is known to be an important contributor to thermoregulation in small marsupial species (eg Fleming, 1980).

The development of an adult Tb rhythm presumably would usually occur some time after the animals leaves the confines of the maternal nest and reaches sexual maturity. This is seen in the rat where a circadian organisation of core Tb is entrained in the young rat once it achieves independent status from its mother (Kittrell and Satinoff, 1986). Post-weaned juvenile *M. domestica* did not always exhibit a 24 hour rhythm leading to the speculation that thermoregulation matures fully well after separation from the mother. Two weaned animals did show a 24-hour rhythm of Tb atypical of adults indicating that in many cases the development of a regular Tb rhythm occurs once independence from the mother is achieved in *M. domestica*. The development of thermoregulation in marsupials is still not fully understood although it is usually established by the time weaning occurs (Morrison and Petajan, 1962).

Levels of activity are often discussed simultaneously with changes in core Tb. Tb rhythms have been correlated with activity levels in rodents (DeCoursey et al., 1998) and in marsupials such as *Antechinus stuartii* (Kortner and Geiser, 1995) and the hairy-nosed wombat, *Lasiorhimus latifrons* (Wells, 1978). During light phases low Tbs are generally observed in *Antechinus* with higher temperatures maintained during the dark phase. Single peaks in Tb approximately halfway through the light phase also correspond to bursts in activity in *A. stuartii* (Kortner and Geiser, 1995). It is highly possible therefore that peaks in Tb observed in *M. domestica* in the nocturnal phase are related to activity. According to Gordon (1993b), the amplitude of the circadian temperature rhythm in rats is internally set by the central nervous system however motor activity can increase its amplitude during evening hours. Unfortunately, activity levels were not measured in this study but general observations of animals made during daylight and nocturnal hours indicate some relation between activity and Tb, similar to that reported in rats.

Observations from this study indicate that *M. domestica* in a thermal gradient selected ambient temperatures more freely during nocturnal hours (see Chapter 6) indicating a higher level of activity during this time period. This is typical of a nocturnal mammalian species and has previously been observed in free-living kangaroos such as the red kangaroo (*Macropus rufus*) (McCarron et al., 2001).

Rhythms of Tb observed in marsupials have been found to be either monophasic or biphasic. A biphasic rhythm of Tb has been observed in the wombat, *V. ursinus* (Peters and Rose, 1979) and biphasic patterns also appear evident in previous studies with *P. breviceps* (Dawson and May, 1984) and *Antechinus stuartii* (Kortner and Geiser, 1995). Aschoff (1966) suggested that the basic two peak pattern of locomotor activity is a persistent property of the mammalian circadian oscillating system. Pickard et al., (1984) suggests that the body temperature rhythm in some animals is split in association with the splitting of the circadian rhythm of locomotor

activity. Rhythms of locomotor activity affect Tb rhythms in the golden hamster (Brown and Refinetti, 1996) and activity and Tb rhythms in the tree shrew, *Tupaia belangeri* are synchronised with a bimodal Tb rhythm exhibited (Refinetti and Menaker, 1992a).

However, nycthemeral cycles of Tb in most marsupials appear to be unimodal (Morrison, 1965, Guiler and Heddle, 1974; Brown and Dawson, 1977; Halse and Rose, 1988; Rose et al., 1990). This typical monophasic pattern is also evident adult *M. domestica* and, although activity rhythms were not determined in this study, general observations indicate a synchronisation of activity with Tb levels. Activity is related to heat production and therefore it is plausible that some correlations between the two processes would exist. Metabolic heat production and its regulation are the underlying processes that change heat content and therefore drive deep body temperature. In humans, circadian changes in Tb can be explained by heat regulatory processes as a natural consequence of day-night changes in activity levels (Webb, 1995). Similar such changes can therefore be explained in marsupial species resulting in either a biphasic or monophasic pattern of core Tb regulation.

The adult male *M. domestica* utilised in this study were relatively old which may have influenced Tb rhythms. Aged rats often exhibit reduced amplitudes in the circadian thermoregulatory system (Aschoff, 1966; Sacher and Duffy, 1978; Refinetti et al., 1990; Shemi and Kaplanski, 1995). As well as a weakening in amplitude, shortening in period and advancement in phase with desynchronisation has also been reported with aging (Myers and Badia, 1995). Aging is believed to be responsible for disruptions in the rhythmic and homeostatic components of the thermoregulatory system (Mailloux et al., 1999) however rats of the same age may differ in robustness and stability of their circadian rhythms of Tb (Li and Satinoff, 1995). Such individual differences were apparent in *M. domestica*. It is possible though that gradient animals exhibited greater amplitudes in Tb as they were able

to utilise thermoregulatory behaviour to overcome any natural weakening effect as a result of age.

Circadian rhythms of Tb are not observed in juvenile *M. domestica* and refinement of the circadian phase of Tb appears likely as they develop their full thermogenic capabilities. In comparison to the older *M. domestica* used in this study, there appears significant differences between circadian patterns of Tb in the two age groups as older animals do exhibit a typical 24-hour rhythm of Tb. There appears to be no reduction in amplitude of Tb rhythms in aged *M. domestica* unlike that observed in aged rats and a reduction in phase rather than an advancement is sometimes observed. These findings indicate that aging does not adversely affect circadian rhythms of Tb in *M. domestica*. However, the animals used in this study were sourced from a laboratory colony and may not truly represent the effects of aging seen on this species in its natural habitat.

The colony of *M. domestica* was maintained at a relatively constant Ta (ie. 28-30°C) and exposure to a range of Tas in the thermal gradient did lead to some changes in Tb patterns recorded. Although not markedly different, the different Tbs observed in adult *M. domestica* in a thermal gradient does indicate some role of Ta in marsupial thermoregulation. This role is obviously generically absent in the colony as a whole but can be triggered when different Ta options are presented to animals. Ambient conditions can be utilised to avoid excess elevations or decreases in core Tb which is apparent in this study as the variance in Tb was reduced in gradient animals. Although this change in variance did not result in significant differences in Tb rhythms it does indicate that marsupials can select Tas to adjust their Tb and increase their thermoregulatory efficiency as behavioural control is less energy-demanding than autonomic control of thermogenesis. Autonomic effectors are established as the primary effectors in mammalian thermoregulation (eg Gordon, 1994) however it is becoming more apparent that behaviour with respect to Ta selection does influence Tb rhythms. Thermoregulatory behaviour was subsequently studied in *M. domestica* in

this study to ascertain the relative effects of behaviour, if any, in marsupial thermogenesis. In addition, as the *M. domestica* used in this study were warm-acclimated laboratory animals, the ability of these animals to adapt to the cold is of interest.

As hypothesised, Tb rhythms are clearly demonstrated over a 24-hour period in adult laboratory-bred *M. domestica*. However, such a rhythm is not always apparent in juvenile animals despite similar mean 24-hour Tb to adults. Core Tb is more variant in juvenile animals while adult animals allowed to select preferred Ta over 24-hour periods show the least variance in core Tb. This latter observation indicates that the environment has a key influence on the maintenance of core Tb.

CHAPTER 4

THERMOREGULATORY RESPONSES TO COLD AMBIENT TEMPERATURES

4.1 Introduction

Ambient temperature is known to have significant effects on the circadian rhythms of mammalian species (eg Francis and Coleman, 1997). Thermoregulatory responses to extreme temperatures have been widely investigated in various mammalian species including marsupials (eg Scholander et al., 1950; Wallis, 1979). Various studies have compared the thermoregulatory efficiencies of warm-acclimated and cold-acclimated animals when exposed to a variety of ambient conditions (eg Dawson and Olson, 1988). In particular, smaller marsupial species have been studied and factors of insulative properties, the ability to enter torpor and non-shivering and shivering thermogenesis mechanisms have been investigated (Bartholomew and Hudson, 1962; Wallis, 1979, Smith and Dawson, 1984a and 1984b; Geiser, 1988). Many studies however have failed to consider the effects of extreme Ta on the circadian rhythm of Tb.

Cold ambient temperatures increase the energy demands of thermoregulation in mammals (Dauncey, 1990). Small dasyurid marsupials have been shown to maintain levels of metabolism similar to eutherians of comparative size when exposed to the cold (Dawson and Dawson, 1982). Increases in core Tb have been observed in response to the cold in a number of marsupials including Tasmanian devils (Nicol and Maskrey, 1980), bettongs (Rose et al., 1990) and wombats (Wells, 1978). However, Smith and Dawson (1984a; 1984b) observed no significant Tb change in *Dasyuroides byrnei* when exposed to the cold for prolonged periods of time.

NST occurs in BAT in small or newborn mammals where uncoupling protein (UCP) is used to generate heat rather than adenosine triphosphate (ATP) (Trayhurn, 1993). Marsupials appear to lack BAT (eg Wallis, 1979) although it has been reported to exist in small amounts in *S. crassicaudata*; by using an antibody to UCP (Hope et al., 1997). However, its relative lack of evidence in many marsupial species (Hayward and Lisson, 1992) does indicate its likely absence. As a result, many marsupials cannot rely on NST to generate heat when environmental conditions are unfavourably cool. Noradrenaline is the principal mediator of NST and has been shown to mediate NST in macropod marsupials (Nicol et al., 1997; Rose et al., 1999) presumably by acting on skeletal muscle as proposed by Ye et al., (1995).

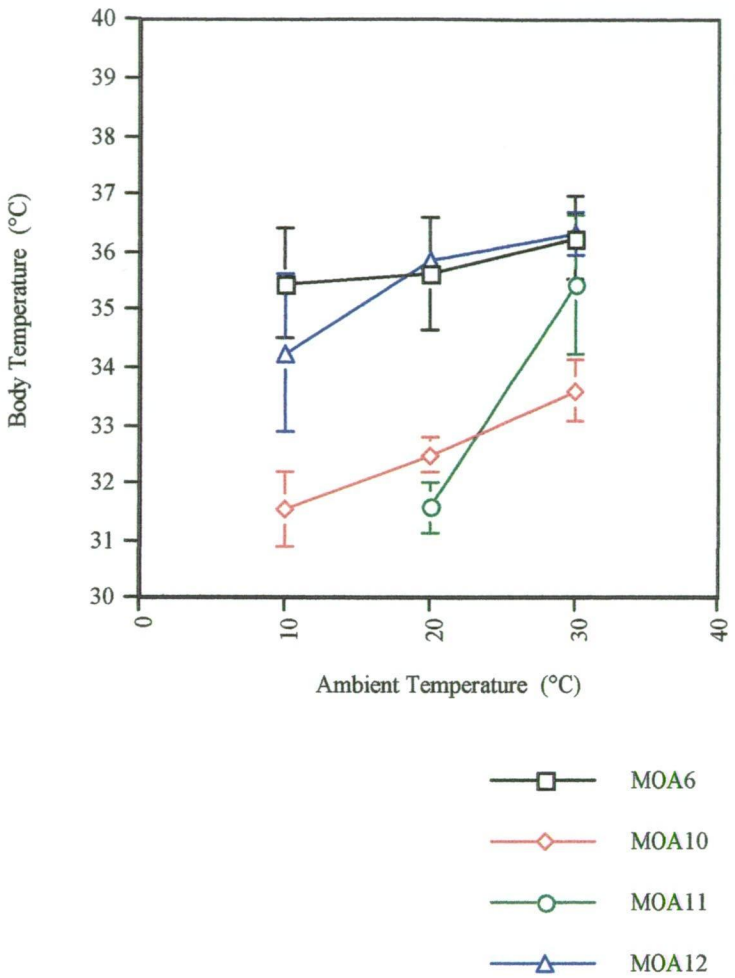
Heat production is a key mechanism in non-macropod marsupials as observed in warm-acclimated *D. byrnei* when exposed to ambient temperatures as low as -5°C (Smith and Dawson, 1985). Similarly, warm-acclimated *M. domestica* maintain Tb at ambient temperatures as low as 5°C through increased heat production and reduced conductance (Dawson and Olson, 1988). However, warm adapted *Antechinus stuartii* are unable to maintain a steady core Tb in the cold (Reynolds and Hulbert, 1982).

The colony of *M. domestica* used in this study were laboratory bred in an environment maintained at 28-30°C. Thus, it was of interest to determine how well these animals could thermally adapt to exposure to cooler ambient temperatures for a period of a few days. It was hypothesised that animals would not adapt well to colder ambient conditions due to their heat-acclimated states from birth and consequently would not be able to maintain a steady core Tb. However, a subsequent exposure to colder Ta would show an adjustment in the thermal state of the animal resulting in Tb maintenance and indicate a degree of acclimation to colder temperatures.

4.2 Results

Continuous Tb measurements were recorded from four adult *M. domestica* while exposed to Tas of 30°C, then 20°C and finally 10°C. MOA6 was the initial subject and was first exposed to 30°C for ten days followed by exposure to 15°C for seven days as a pilot experiment. This was because none of the animals from the colony (from which the animals were obtained) had been exposed to Tas below approximately 28°C (ie warm-acclimated). Mean Tb was calculated at 15 minute intervals over 24 hour periods and circadian patterns of Tb determined using Fourier analysis. The mean daily Tb was also determined for each animal at each Ta as shown in Table 4.2.1. Figure 4.2.1 illustrates the mean daily Tb at each Ta and shows some differences between Tbs of individual animals at various cold temperature exposures.

Figure 4.2.1 Mean Body Temperature of four individual *M.domestica* at ambient temperatures of 10°C, 20°C and 30°C
(means ± SD were calculated over a number of days at each ambient temperature)



Ambient temperature had a profound effect on Tb rhythm as illustrated in the typical recordings from MOA12 in Figures 4.2.2(a-g) and the continuous measurements of Tb at each Ta for each animal shown in Appendix 4. Tbs were analysed by single series Fourier analysis and results plotted by period using transformations in which the mean was subtracted and detrended (using StatSoft STATISTICA 4.1). The effect of Ta on Tb is even more apparent in these Fourier transformation plots as shown for each animal in Appendix 9. Appendix 9 also tables the period values for each Fourier analysis of individual animals at each Ta. Due to the obvious differences observed between individuals, the results obtained were considered separately for each animal with respect to the presence of circadian cycles.

Figure 4.2.2a Typical Continuous Recording from an adult *M. domestica* (MOAC12) exposed to a Ta of 30°C for the first time

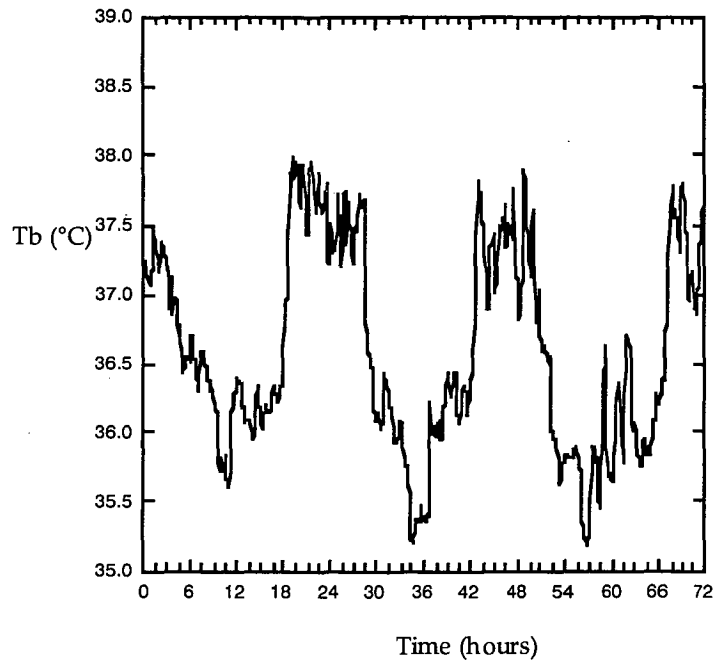


Figure 4.2.2b Typical Continuous Recording from an adult *M. domestica* (MOAC12) exposed to a Ta of 20°C for the first time

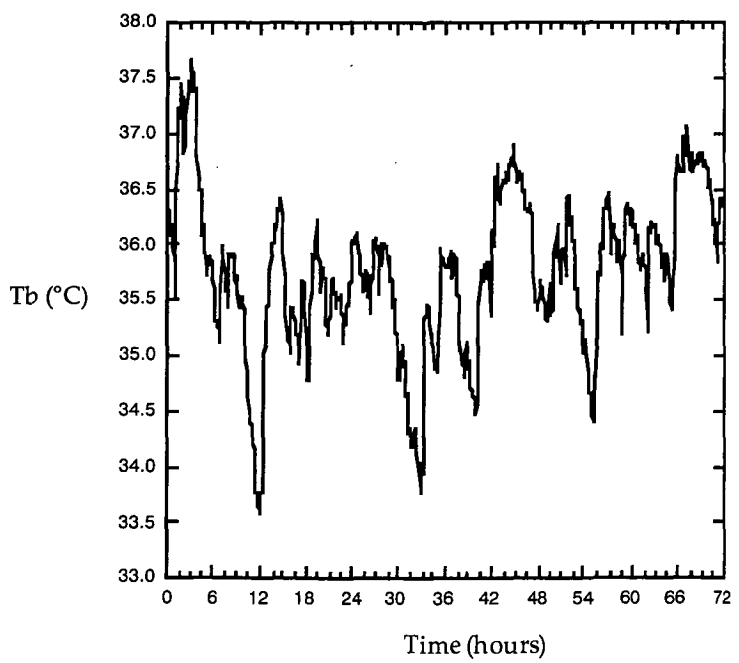


Figure 4.2.2c Typical Continuous Recording from an adult *M. domestica* (MOAC12) exposed 10°C for the first time and then exposed to 30°C for the second time

*Ta changed from 10°C to 30°C

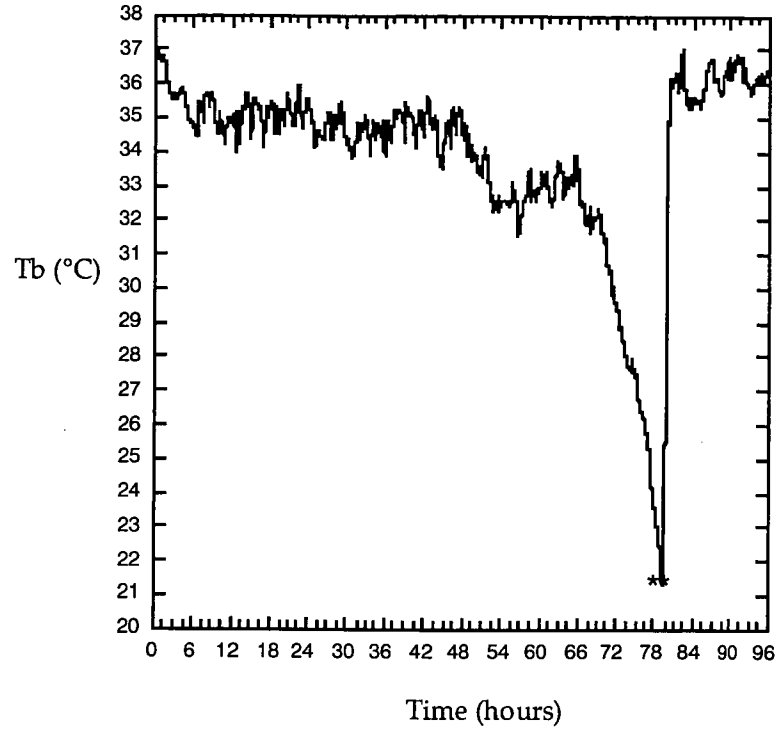


Figure 4.2.2d Typical Continuous Recording from an adult *M. domestica* (MOAC12) during a second exposure to 30°C

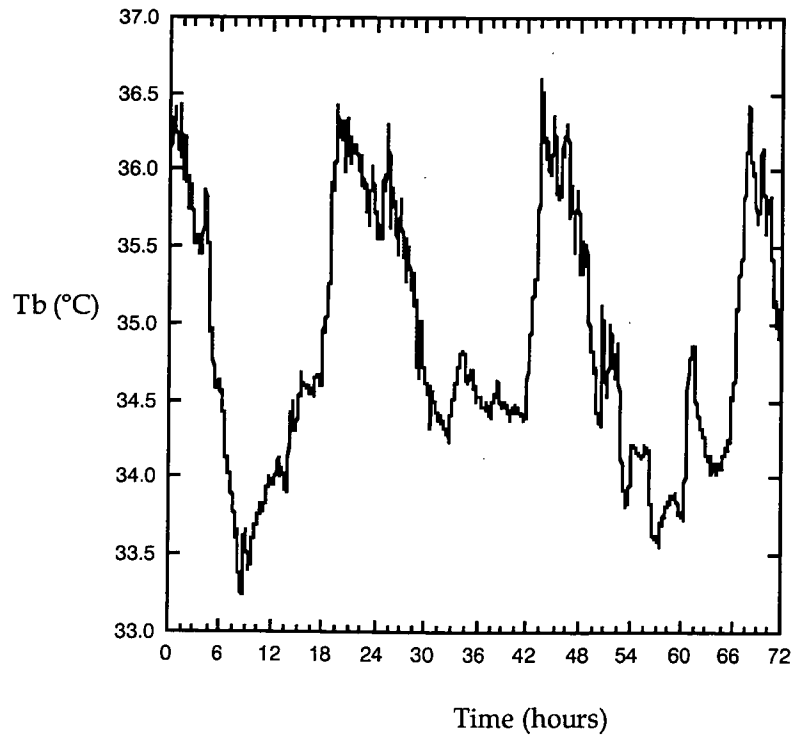


Figure 4.2.2e Typical Continuous Recording from an adult *M. domestica* (MOAC12) exposed to a Ta of 20°C for a second time

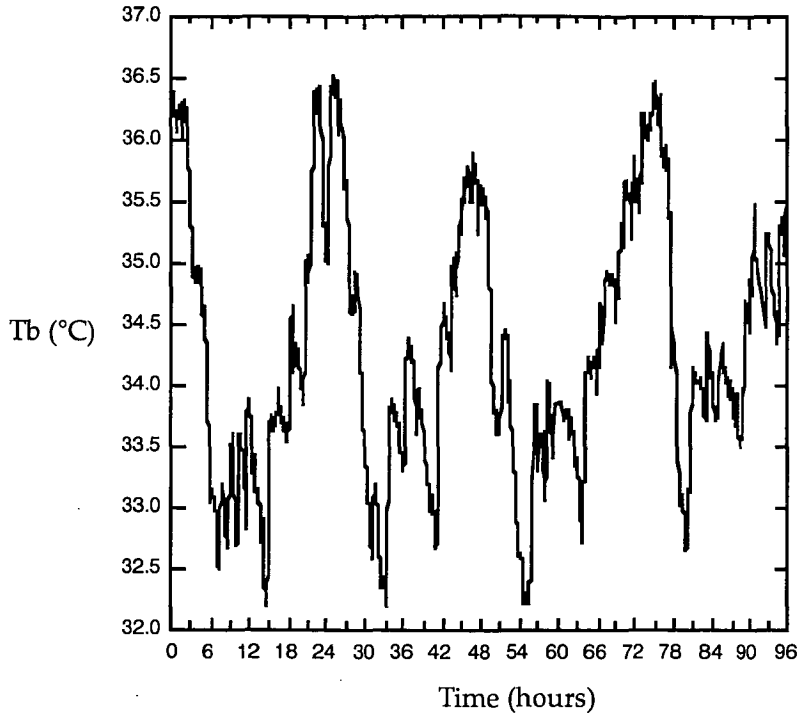


Figure 4.2.2f Typical Continuous Recording from an adult *M. domestica* (MOAC12) exposed to a Ta of 20°C (for a 2nd time) and 30°C (for a 3rd time)

* Ta changed from 20°C to 10°C ** Ta changed from 10°C to 30°C

[• no Tb recorded due to technical problems]

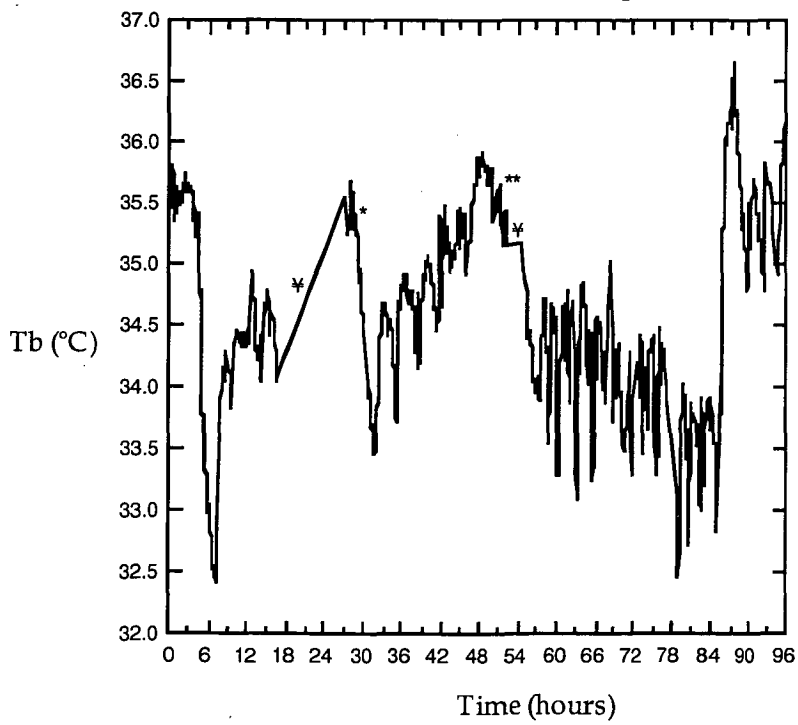
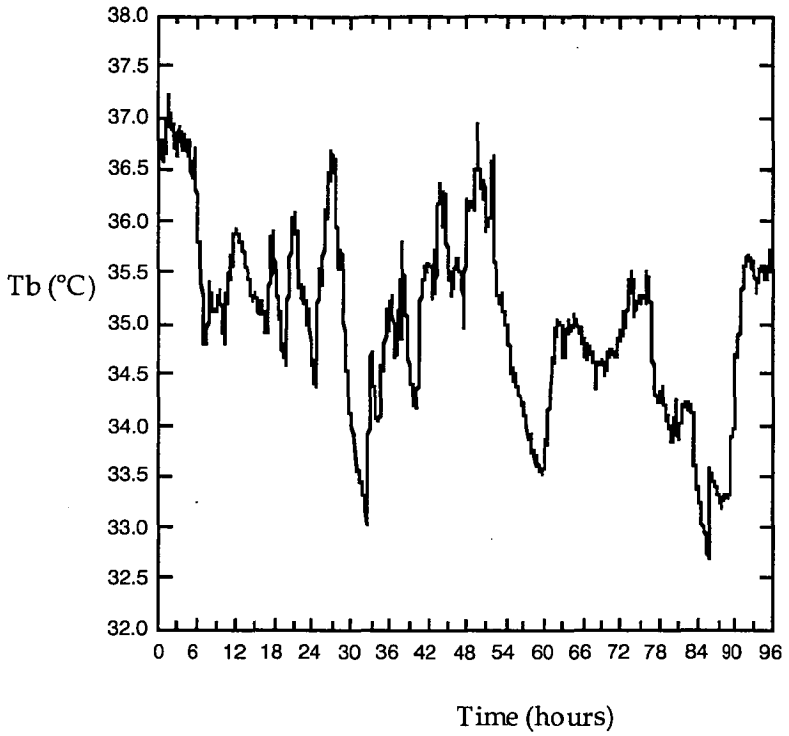


Figure 4.2.2g Typical Continuous Recording from an adult *M. domestica* (MOAC12) exposed to a Ta of 30°C for a third time



Means (X±SD) of Tb at 15 minute intervals were determined for all four *M. domestica* and were pooled for statistical analysis using ANOVA. No significant differences were found between Tbs recorded at different Tas however significant differences were found between Tb of individual animals at first exposure to 20°C ($F=36.3$; $p<0.05$). In addition, significant differences between individual animal Tbs were found when comparing first exposures to 20°C and 10°C ($F=27.6$; $p<0.05$) and first exposure to 10°C and second exposure to 10°C ($F=310.5$; $p<0.05$). These statistics are limited by the small sample number used in this study. Tb results from individual animals and the group as a whole were therefore considered to determine the effects of cold ambient temperatures on mean Tbs and Tb rhythms in warm-acclimated *M. domestica*.

Table 4.2.1 Daily core body temperatures (Tb) of *Monodelphis domestica* as determined from continuous recordings over successive 24 hour periods at different ambient temperatures

Animal	No. of days analysed	Ambient Temperature (°C)	Body Temperature (°C) (Mean±SD)
MOAC6	10	30	35.6±0.3
	7	15	35.1±1.2
	9	30	36.2±0.7
	22	20	35.6±1.0
	14	10	35.5±1.0
	54	30	35.8±0.8
	6	10	34.3±0.6
	3	30	36.0±0.6
MOAC10	13	30	33.6±0.5
	8	20	32.5±0.3
	4	10	31.5±0.6
	27	30	33.8±1.1
	7	20	32.3±3.5
	1	10	30.2±1.1
	14	30	34.4±0.8
MOAC11	14	30	35.4±1.2
	4	20	31.6±0.4
MOAC12	14	30	36.3±0.4
	10	20	35.9±0.3
	3	10	34.2±1.4
	18	30	36.4±0.9
	7	20	35.7±0.4
	1	10	35.9±0.7
	18	30	36.3±0.5

Appendix 9 illustrates the Fourier analysis of Tb at each Ta for MOAC6, MOAC10, MOAC11 and MOAC12. Tables 4.2.2a-4.2.2d show the amplitude, acrophase (timing of peak) and period for Tb at each Ta for each animal. As shown by Table 4.2.2c recordings from MOAC11 were only achieved at 30°C and 20°C. After four days of recording this animal was removed from the constant temperature cabinet as it was noticed that the animal was not particularly responsive and was losing weight rapidly (129g to 120.3g). The animal died (from unknown causes) two days after removal from the temperature cabinet.

Table 4.2.2a Analysis of the effects of Ambient Temperature (Ta) on Core Body Temperature in MOAC6: Effects on amplitude, acrophase, and period

[amplitude is the difference between the maximum and minimum temperature recorded; acrophase is the timing of the largest peak and is shown in 24-hour time; period was determined by Fourier analysis]

Ta (°C)	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
30	32.8	36.0	3.2	13:08	24
15	31.0	35.3	4.3	00:31	24
30	32.5	36.2	3.7	20:40	24
20	31.0	35.7	4.7	23:18	24
10	31.1	35.3	4.2	05:26	12
30	31.1	36.1	5.0	22:40	24
10	30.9	33.7	2.8	21:48	24
30	32.4	36.0	3.6	01:29	24

Table 4.2.2b Analysis of the effects of Ambient Temperature (Ta) on Core Body Temperature in MOAC10: Effects on amplitude, acrophase, and period

[amplitude is the difference between the maximum and minimum temperature recorded; acrophase is the timing of the largest peak and is shown in 24-hour time; period was determined by Fourier analysis]

***Tb recorded for only 24 hours at second exposure to 10°C therefore Fourier analysis irrelevant**

Ta (°C)	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
30	31.6	36.2	4.6	07:11	24
20	30.3	34.7	4.4	21:13	12
10	28.4	34.0	5.6	02:47	48
30	30.1	35.3	5.2	21:13	24
20	30.2	34.0	3.8	22:26	12
10	21.0	31.7	10.7	10:03	24*
30	30.0	34.9	4.9	21:42	24

Table 4.2.2c Analysis of the effects of Ambient Temperature (Ta) on Core Body Temperature in MOAC11: Effects on amplitude, acrophase, and period

[amplitude is the difference between the maximum and minimum temperature recorded; acrophase is the timing of the largest peak and is shown in 24-hour time; period was determined by Fourier analysis]

Ta (°C)	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
30	32.7	36.8	4.1	19:51	24
20	28.4	33.6	5.1	12:13	72

Table 4.2.2d Analysis of the effects of Ambient Temperature (Ta) on Core Body Temperature in MOAC12: Effects on amplitude, acrophase, and period

[amplitude is the difference between the maximum and minimum temperature recorded; acrophase is the timing of the largest peak and is shown in 24-hour time; period was determined by Fourier analysis]

***Tb recorded for only 24 hours at second exposure to 10°C therefore Fourier analysis irrelevant**

Ta (°C)	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
30	33.0	37.0	4.0	21:48	24
20	33.6	37.7	4.1	02:57	12
10	29.4	35.6	6.2	18:27	24
30	32.9	37.2	4.3	19:35	24
20	32.2	36.5	4.3	01:04	24
10	32.5	35.0	2.5	20:17	3.4*
30	32.7	37.3	4.6	01:29	24

Cold ambient conditions had a profound effect on all four animals studied. Significant differences were found between circadian Tb at 30°C and 20°C ($t=20.0$; $p<0.0005$: $t-=2.1$; $p<0.05$), 30°C and 10°C ($t=44.4$; $p<0.0005$: $t=-10.7$; $p<0.0005$), 20°C (first exposure) and 20°C (second exposure) ($t=-18.1$; $p<0.0005$) and 10°C (first exposure) and 10°C (second exposure) ($t=-79.6$; $p<0.0005$). Circadian rhythms of Tb in all four animals were affected by reductions in ambient temperature. At initial exposures to 30°C all four individuals exhibited a Tb rhythm which was equal to 24 hours. Some changes to this rhythm occurred at 20°C, however the type and extent of these changes differed in individual animals. At a Ta of 10°C, a rhythm between 12 and 48 hours was observed. A change of Ta back to 30°C then resulted in an initial disruption of the Tb rhythm which was eventually restored to a 24-hour cycle. In MOAC6 a change in Ta from 30°C to 15°C resulted in change in the circadian pattern of Tb from a monophasic pattern

to a biphasic pattern (with periods at 12 and 24 hours). This phenomenon was not seen at any other Ta or in any other animal in this study.

Repeated exposures to lower Tas provided compelling results. MOAC6 exhibited a 24-hour rhythm of Tb when exposed to 10°C for a second time despite a disruption to Tb rhythm at this Ta during the first exposure period. In two animals (MOAC10 and MOAC12) in which a second exposure to 20°C, and subsequently, 10°C were investigated, interesting observations were made. MOAC12 maintained a 24-hour Tb rhythm at a second exposure to 20°C despite exhibiting a 12 hour rhythm when first exposed to this Ta. This animal then showed no distinct Tb rhythm at 10°C despite exhibiting a 24-hour rhythm during its first exposure to 10°C. MOAC10 however, showed a 12-hour Tb rhythm at a Ta of 20°C (during both the first and second exposures) and a rhythm of 24 hours at its second exposure to 10°C (compared to a 48-hour rhythm of Tb during first exposure to this low Ta). When the Ta was then set to 30°C, both animals exhibited a slight disruption in Tb rhythm but a rhythm of 24-hours was restored in both cases and in a shorter time than after the initial exposure periods to the lower Tas in the case of MOAC10.

4.3 Discussion

The Tb responses to cooler temperatures observed in warm-acclimated *M. domestica* show how critical Ta is to Tb maintenance. Each individual animal exhibited significant changes in circadian Tb when exposed to a Ta below 30°C (ie below the acclimation temperature). This indicates that warm-acclimated *M. domestica* respond to Ta by adjusting their set-point of thermoregulation. This was also suggested by Gwosdow and Besch (1985) who proposed that the acclimation temperatures of rats affects the TNZ and consequently alters the hypothalamic set-point temperature. The *M. domestica* used in this study were from a laboratory-bred colony maintained at a constant room temperature of 28-30°C. These animals were therefore warm-acclimated and so they may have adjusted

their hypothalamic set-point of thermoregulation accordingly. Alternatively, the acclimation of *M. domestica* may affect their thermal performance rather than the set-point.

When exposed to lower Tas warm-acclimated *M. domestica* decrease their core Tb to accommodate for the change in ambient conditions. This has been observed previously in rats (Sugimoto et al., 1999) but is in contrast to previous findings in *M. domestica* by Dawson and Olson (1988). Dawson and Olson (1988) found that warm-acclimated *M. domestica* (acclimated to 25-27°C) showed considerable thermogenic ability to adapt to temperatures as cold as 5°C for approximately 4 hours, although it was noted that some opossums did die at this extreme Ta. This indicates a short-term adaptation to the cold involving reduced conductance and increased heat production which may be insufficient to fulfil the long-term thermal adaptation to cold investigated in this study. Heat production appears to be a key mechanism in cold adaptation in marsupials and would involve shivering thermogenesis, NST and other metabolic activity. It was noted that all animals increased appetite as ambient temperature decreased suggesting that metabolic heat production was a crucial mechanism in the maintenance of Tb. However, this investigation offers no conclusive evidence as to what mechanisms may have been used in warm-acclimated *M. domestica* in order to regulate Tb. Smith and Dawson (1985) observed higher levels of heat production in cold-acclimated *D. byrnei* compared to warm-acclimated animals when exposed to very low Tas for 2.5 hours. This also suggests that warm-acclimated marsupials have a reduced competence for cold adaptation.

It is well known that eutherian mammals are able to acclimatise to cold temperatures by elevating their potential for NST thus resisting the limiting factors imposed by chronic cold exposure (Jansky, 1973; Kuroshima, 1993; Dicker et al., 1995). The inability of *M. domestica* to cope with long-term exposures to the chronic cold suggests that NST may be absent in this group of warm-acclimated animals. The absence of NST has been reported

in the small marsupial *A. stuartii* (Reynolds and Hulbert, 1982) but has been found to occur mediated by noradrenaline in the potoroo, *P. tridactylus* (Nicol, 1978). According to Nicol et al., (1997) noradrenaline-mediated NST is restricted to macropod marsupials which is supported by recent evidence of noradrenaline-mediated NST in *B. gaimardi* (Rose et al., 1999). Smith and Dawson (1985) have suggested that endocrine factors such as thyroid hormones, corticosteroids and adrenalin may play a role in marsupial NST. The status of NST in marsupials remains controversial and this study offers no strong evidence of NST in *M. domestica*. The major site of NST in eutherian mammals is BAT (brown adipose tissue) and it has been suggested by Hayward and Lisson (1992) that BAT is absent in marsupial species (although their study did not include all species including *M. domestica*). This lack of BAT in marsupials remains an area of controversy with evidence for BAT reported in *S. crassicaudata* (Hope et al., 1997). It may be possible that other anatomical sites such as skeletal muscle are involved in marsupial NST as observed in ducklings (Duchamp and Barre, 1993) and small mammals (Dubois-Ferriére and Chinet, 1981). Based on observations in *B. gaimardi*, Ye et al (1995) have suggested that marsupial skeletal muscle may contribute to whole body thermogenesis moreso than skeletal muscle in eutherian species. The role of NST and indeed BAT in marsupial thermogenesis remains one of great debate and this study although providing no conclusive evidence for either argument does highlight the need for future studies in this area of great controversy.

Due to the circadian variations of Tb in *M. domestica*, and marsupials generally, a true picture of cold (or warm) adaptation can only be realised when an animal is exposed to extreme conditions for a period of at least 24 hours. Many studies such as Smith and Dawson (1985) and Dawson and Olson (1988) have only considered thermogenic responses in marsupials to short-term exposures to very cold temperatures. This data becomes very limited in addressing overall thermoregulatory mechanisms to the cold when considering the typical fluctuations observed in marsupial Tb throughout a daily cycle. In addition, exposures to cold temperatures in the

natural habitat are more likely to be of longer duration than just a few hours. When exposed to 10°C *M. domestica* initially managed to maintain Tb at a relatively normal level, however after a short period (of a few hours) Tb decreased significantly in response to the consistently cold Ta. This supports the observations of Dawson and Olson (1988) but indicates strongly that long-term exposures to cold Ta can not be tolerated by these warm-acclimated animals. The Ta of an endotherm's natural habitat enables the animal to thermally adjust to varying temperature using physiological and behavioural mechanisms. Such attunement of mechanisms are lacking in animals acclimatised to a relatively constant Ta resulting in deficiencies in thermogenic capabilities.

It has previously been suggested that mammalian core body temperatures in mammals rarely decrease in exposure to relatively low Tas as thermoregulatory mechanisms, ie heat production occurs to maintain Tb (Banin et al., 1994; Gordon, 1993b). However, different responses (both behavioural and physiological) to varying Ta have been observed in different rodent species (Oufara et al., 1987; Ishii et al., 1996) which are likely attributable to different thermogenic mechanisms being utilised to maintain Tb. Yet, according to the findings of this study a significant drop in Tb can occur in South American opossums when environmental temperatures decrease. This can be explained by the acclimation of these animals to a relatively warm and constant external environment since birth. This will have limited the ability of the animals to develop thermoregulatory mechanisms to adapt to changing ambient conditions. It is obvious that a larger range of Ta experienced by an endotherm in a natural environment enables the animal to thermoregulate more effectively using physiological processes when exposures to extreme temperatures are experienced. The animals used in this study could utilise physiological processes such as increased heat production to maintain Tb for a short time in response to the cold. However, due to the conditioning of the thermoregulatory processes in these animals to warm environments only, long term tolerance to cold Tas could not necessarily be maintained.

The inability of warm-acclimated *M. domestica* to thermoregulate effectively in a cold environment was readily observed in the dramatic change to the normal circadian pattern of Tb. A change in Ta from 30°C to 15°C resulted in a change from a monophasic Tb pattern to a biphasic Tb cycle in one animal. This dramatic change in cyclic variations of Tb is due to the profound difference between the two Tas and indicates some adaptive abilities in thermoregulation as well as highlighting the importance of the environment in thermogenesis.

Changes in Tas of 10°C and 20°C did produce different Tb results in individual animals however some common effects are apparent from the limited data collected in this study. *M. domestica* appear to maintain their Tb rhythm as a 24-hour cycle for at least a short time period at low ambient temperatures. It is also apparent that an exposure to a lower than normal Ta (ie 20°C) followed by an exposure to an even lower Ta (ie 10°C) does provide these animals with some opportunity to adjust and prepare their thermoregulatory system for cold environments. This adjustment to thermogenesis is essential and presumably must also involve an adjustment of the control system, ie the thermoregulatory set-point proposed to be located in the hypothalamus (eg Osborne and Refinetti, 1995) and various heat-gaining processes. This adjustment to the set-point of thermoregulation is proposed to occur as all animals showed initial disruptions to Tb rhythms when returned to a Ta of 30°C after exposure to colder temperature environments. This disruption involved the re-setting of the hypothalamic set-point to a normal homeostatic range and indicates that while changes to thermogenesis are essential, the control system is also affected.

Repeated exposures to lower ambient temperatures produced interesting results in *M. domestica*. In one animal (MOAC12) an adjustment to a Ta of 20°C upon second exposure was apparent as a 24-hour Tb rhythm was maintained. A second animal (MOAC10), however, showed a

thermoregulatory adjustment to a Ta of 10°C again through the maintenance of a 24-hour Tb rhythm. Both animals spent 7 days at 20°C and one day at 10°C so the time of exposure to the Ta cannot be considered as a major factor for these differences between animals. However, the animal best adapted to a Ta of 20°C did spend longer exposed to 20°C during the first exposure period. Similarly, the animal able to maintain a 24-hour rhythm at 10°C spent longer at that Ta during the initial exposure period. This is also apparent when considering the results of a third animal (MOAC6) in which a 6 day exposure to 10°C had no effect on the regular 24-hour rhythm of Tb despite no rhythm of Tb at an initial exposure to a Ta of 10°C for 14 days. Time factors therefore may play an important role in allowing an animal to adjust to an extreme change in Ta by desensitising the hypothalamic thermoregulatory system and allowing heat loss and heat production mechanisms to be modulated. Conductance, evaporative heat loss and heat production all significantly increase with a concurrent unchanged Tb in *D. byrnei* when exposed to the cold for a prolonged period (Smith and Dawson, 1984). The limited data collected in this study are certainly supportive of such a theory and is applicable as it provides a mammal with the ability to cope at lower Tas if subsequent exposures do occur. This may be required for the ultimate survival of the species.

Of further consideration in relation to the results obtained for *M. domestica* is the age of the animals used. Biological aging has been shown to impair thermoregulatory mechanisms in the cold due to an increased heat loss and decreased heat production (Florez-Duquet and McDonald, 1998) although the precise mechanisms involved are yet to be defined. The animals used in this study were all considered to be aged as they were beyond typical reproductive age and the oldest animals in the established laboratory colony. These animals were chosen as they were no longer required for breeding purposes and due to quarantine regulations females could not be removed from the colony. Reductions in insulative properties of body fat, reactive tone of cutaneous musculature, skeletal muscle mass, and metabolic activity may be partially or totally involved in the impaired

tolerance to the cold observed. The effects of aging on physiological mechanisms such as thermogenesis have not been previously investigated in marsupials but it is evident from this study that mechanisms may well become less effective with aging.

As hypothesised, the warm-acclimated *M. domestica* investigated in this study did show some difficulties in thermogenic adaptation to the cold (which may or may not be age-related). However, thermoregulatory adjustments were noted in subsequent exposures indicating that thermoregulation in marsupials, and mammals generally, relies on thermal experiences as well as in-built physiological mechanisms. The environmental temperature, although not necessarily a key factor in the ability to thermoregulate, may modify adaptive thermogenic capabilities despite initial disruption in circadian cycles of Tb. Another modifier of thermoregulation and a key regulator of mammalian thermoregulation are the thyroid hormones. The thyroid status of marsupials has been investigated in some species such as *M. eugenii* (Kaethner and Good, 1975), *P. tridactylus* (Nicol, 1977), *I. macrourus* (Hulbert and Augee, 1982), *Tachyglossus aculeatus* (Hulbert and Augee, 1982) and *A. stuartii* (Withers and Hulbert, 1988) but is unknown in *M. domestica.*

CHAPTER 5

THYROID ACTIVITY IN *Monodelphis domestica*

5.1 Introduction

Secretions from the thyroid gland are known to influence energy metabolism and consequently body temperature in mammalian species. For example, the thyroid controls variations in metabolic rate during normal rat thermoregulation (Whitaker et al., 1990). Due to their lower metabolic rates it would be expected that marsupials would have much lower blood concentrations of thyroid hormones than their eutherian counterparts. Indeed, total plasma thyroxine concentrations have been found to be much lower in marsupials than in eutherians (eg Setchell, 1974) however, free thyroxine levels (which relates to the physiologically active fraction) is comparable between eutherian and marsupial species (eg Nicol, 1977). Furthermore, according to Hulbert and Augee (1982), thyroid activity is relatively similar in eutherians and marsupials despite marsupials having somewhat lower levels of metabolism.

The thyroid gland secretes thyroxine (T_4) and triiodothyronine (T_3) which principally act to stimulate metabolic rate and promote growth. Acute exposure to cold and regular physical activity have been shown to increase thyroid activity dramatically in association with a large increase in metabolic rate in eutherians (eg Tomasi, 1991) and marsupials (Withers and Hulbert, 1988). Similarly, heat exposure and aging depress thyroid activity in eutherians (Tomasi, 1991) however this has not yet been observed in marsupials.

Total T_4 and total T_3 plasma concentrations in *M. domestica* were investigated in this study and comparisons made to levels previously reported in marsupials. It was hypothesised that total T_4 and total T_3 levels

in *M. domestica* would be lower than the levels of these hormones in similar-sized eutherian mammals. In addition, it was hypothesised that the levels of total T_4 and total T_3 would increase when warm-acclimated *M. domestica* were exposed to cold Ta (ie less than 30°C) for extended time periods.

5.2 Results

A mean plasma concentration of 23.9±1.5nmol/L was recorded for total T_4 and 1.5nmol/L±0.3 for total T_3 in *M. domestica*. Plasma levels of T_4 ranged from 21.6nmol/L to 23.0nmol/L in MOAG (adult males in a gradient), 22.2nmol/L to 23.4nmol/L in MOAC (adult males in the colony), 23.9nmol/L to 25.0nmol/L in MOAF (adult females in the colony) and 24.1nmol/L to 26.8nmol/L in MOJC (juvenile males in the colony) as illustrated in Table 5.2.1.

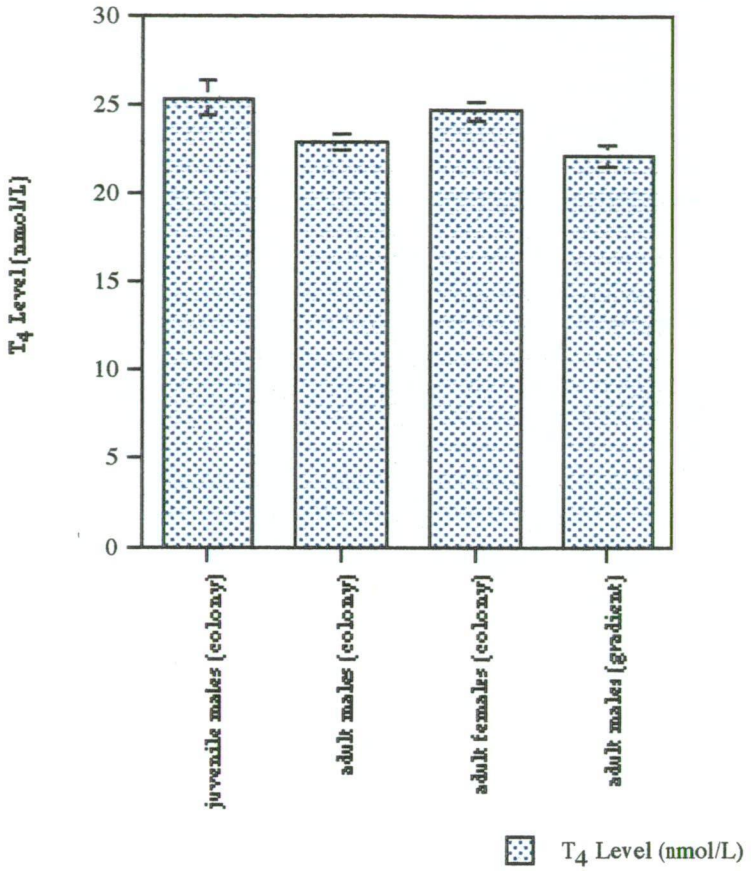
Differences observed between T_4 levels in adult males in the gradient and adult males in the colony were significant ($F=12.0$; $p<0.05$). A significant difference between juvenile and adult plasma T_3 concentrations ($F=24.8$; $p<0.01$) and adult males and adult females in the colony ($F=24.9$; $p<0.01$) was also established. Both juveniles and adult females show similarly high levels of T_4 with no significant difference between the measurements from each group ($F=1.8$; $p>0.05$). Figure 5.2.1 illustrates the T_4 levels measured in all adul;t and juvenile *M. domestica.*

Table 5.2.1 Total plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3) in male and female *M. domestica* maintained at colony temperatures (28°C-30°C) or in a thermal gradient (10°C-40°C)
[MOAC =males maintained in the colony; MOAF = females maintained in the colony; MOAG = males maintained in a thermal gradient]

Animal	T_4 level (nmol/L)	T_3 level (nmol/L)
MOJC2	25.4	1.7
MOJC3	24.1	1.8
MOJC4	25.1	1.6
MOJC5	25.4	1.8
MOJC7	26.8	1.6
MOJC8	26.1	1.8
MOJC9	24.4	1.6
Mean±S.D	25.3±1.0	1.7±0.1
MOAC1	23.4	1.6
MOAC6	23.0	1.6
MOAC10	22.9	1.3
MOAC14	22.2	1.2
Mean±S.D	22.9±0.5	1.4±0.2
MOAF1	24.8	1.4
MOAF2	23.9	1.4
MOAF5	25.0	1.9
MOAF6	24.9	1.7
Mean±S.D	24.6±0.5	1.6±0.2
MOAG13	21.8	0.9
MOAG15	22.0	1.1
MOAG16	21.6	1.0
MOAG17	22.2	1.2
MOAG18	23.0	1.3
Mean±S.D	22.1±0.6	1.1±0.2

Total plasma concentrations of T_3 were also found to be significantly lower in gradient males compared to colony ($F=7.2$; $p<0.05$). Similarly, significant differences were found between T_3 measurements in juveniles and adult animals ($F=12.9$; $p<0.01$) held in the colony. Adult females like juveniles have slightly higher levels of T_3 than adult males in the colony and this difference is significant ($F=6.0$; $p<0.05$). Similarly no significant

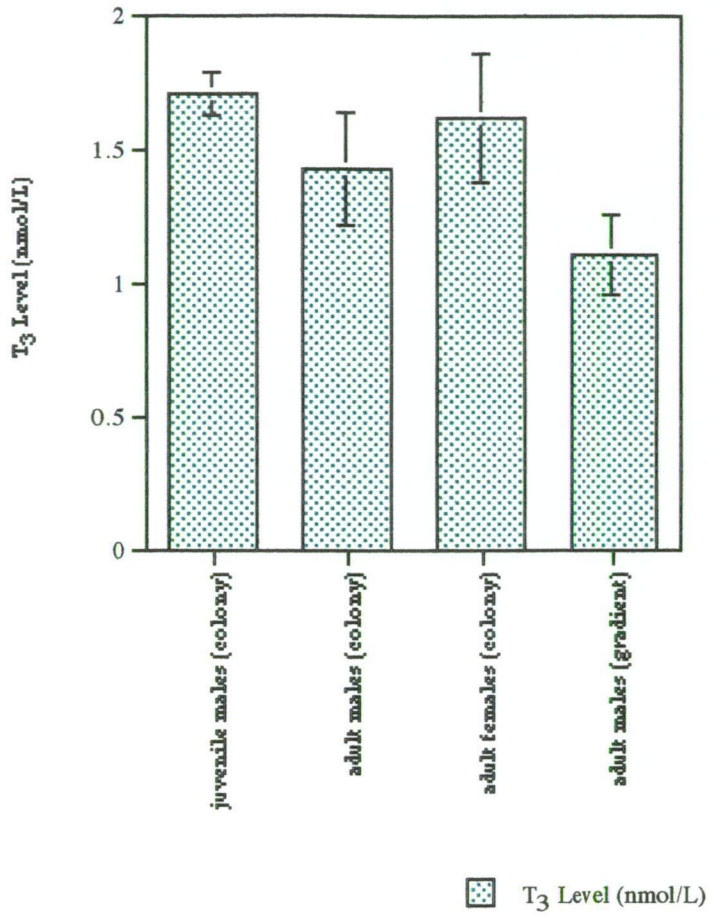
Figure 5.2.1 Total T_4 levels measured in all adult and juvenile *M. domestica* in a colony and adult *M. domestica* while in a thermal gradient



difference is seen between the adult females and juveniles with respect to total plasma T_3 concentrations ($F=0.9$; $p>0.05$).

Total T_3 levels in MOAG ranged from 0.9nmol/L to 1.3 nmol/L compared to 1.2nmol/L to 1.6nmol/L in MOAC. Female *M. domestica* (MOAF) exhibited a range of 1.4nmol/L to 1.9nmol/L and juveniles indicated consistently higher levels of plasma T_3 with a range of 1.6nmol/l to 1.8nmol/L. Table 5.2.1 illustrates relative T_3 measurements for each animal. Figure 5.2.2 illustrates the levels of total T_3 recorded for each group analysed.

Figure 5.2.2 Levels of T_3 in adult and juvenile *M. domestica* in a colony and adult *M. domestica* in a thermal gradient



Plasma concentrations of T_4 and T_3 were determined from blood samples from a single animal, MOAC6 at 10°C and 20°C and are shown in Table 5.2.2. Additional blood samples were not obtained from other animals subjected to cold temperatures due to an inability to successfully obtain blood via cardiac puncture in these animals without killing them. Thyroid hormone levels were noted to increase as environmental temperature decreased in MOAC6. The thyroid hormone concentrations measured at 10°C were higher than those recorded at 20°C and 30°C as illustrated in Figures 5.2.3a and 5.2.3b.

Figure 5.2.3a Effects of different ambient temperatures on plasma concentrations of T_4 in one adult *M. domestica*

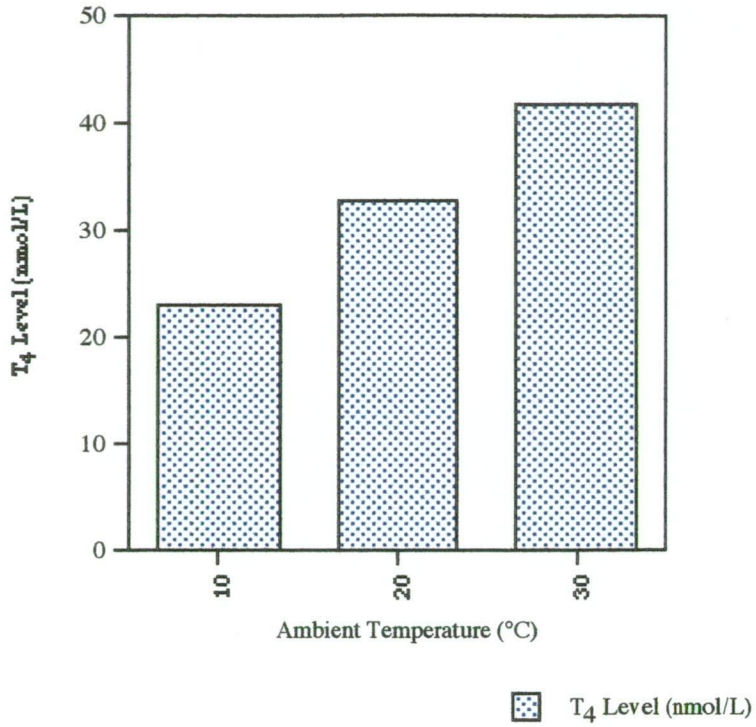


Figure 5.2.3b Effects of different ambient temperatures on plasma concentrations of T_3 in one adult *M. domestica*

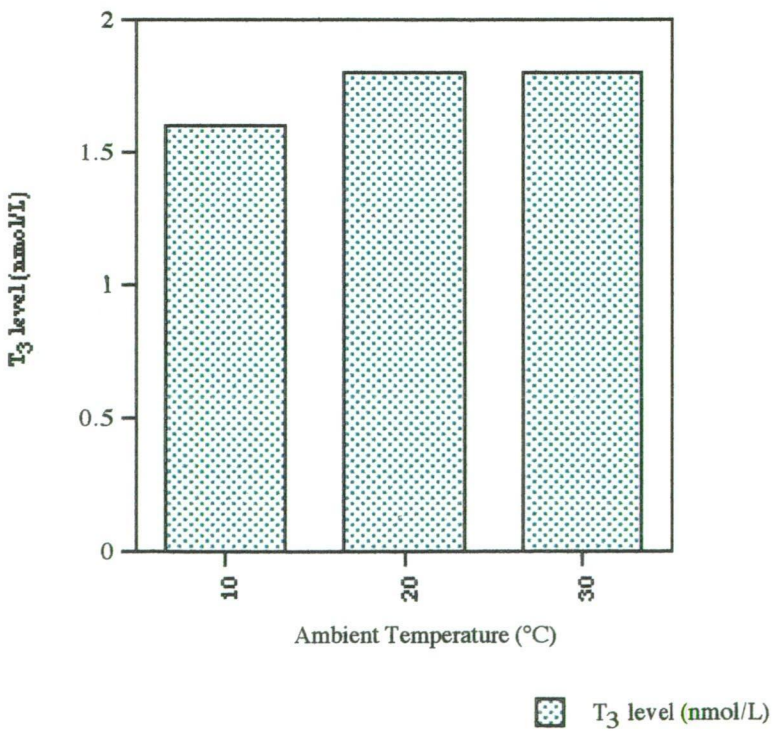


Table 5.2.2 Thyroid hormone levels (T_4 and T_3) in an adult *M. domestica* exposed to environmental temperatures of 30°C, 20°C and 10°C.

Environmental temperature (°C)	T_4 level (nmol/L)	T_3 level (nmol/L)
30	23.0	1.6
20	32.6	1.8
10	41.6	1.8

5.3 Discussion

In this study, total plasma thyroid hormone levels in *M. domestica* were determined by radioimmunoassay methods. These levels were found to be higher than those found, using comparable methods, in the adult tammar wallaby, *Macropus eugenii* (Janssens et al., 1990). Such differences may be explained by the larger size of *M. eugenii* resulting in a lower metabolic rate and therefore a lower level of thyroid activity. As a small-sized marsupial *M. domestica* typically exhibits a relatively high metabolic rate and would be expected to have high blood concentrations of thyroid hormones in accordance with this increased level of energy metabolism. T_4 secretion rates measured in a smaller marsupial species, *Antechinus stuartii* have been shown to increase with increases in energy metabolism (Withers and Hulbert, 1988). The high total T_4 and T_3 levels found in *M. domestica* are therefore not surprising given the small size of this marsupial. Heat exposure has been found to depress plasma thyroid hormone levels in rats (Shido et al., 1993). This does not appear to be the case for warm-acclimated *M. domestica*.

Unfortunately, thyroid hormone levels have not been measured in a similar-sized marsupial species to *M. domestica* although secretion rates have been reported for *A. stuartii* (Withers and Hulbert, 1988). Hulbert and Augee (1982) reported total T_4 levels of 22.0±1.6nmol/L and total T_3 levels of 1.5±0.2nmol/L in the bandicoot, *Isoodon macrourus* and Nicol (1977) reported

total T_4 levels equivalent to 24nmol/L in *P. tridactylus*. Total T_4 levels of 3.2nmol/L and 12.2nmol/l have been measured in *P. cinereus* (Lawson et al., 1996) and *M. eugenii* (Janssens et al., 1990) respectively. Total plasma thyroid hormone levels measured in *M. domestica* therefore correlate fairly well with thyroid hormone concentrations previously measured in other small marsupial species.

Interestingly in this study, there were some differences observed between thyroid hormone levels (particularly thyroxine) measured in animals maintained at a constant temperature in the colony and animals exposed to a gradient of temperatures which enabled them to behaviourally seek environmental temperatures according to their thermoregulatory requirements. This is despite no statistical difference between daily body temperatures of these animals (see Table 3.2.3). It is possible that the gradient animals chose to use temperature seeking behaviour to modulate their body temperature thus reducing the necessity to rely on thyroid hormones to modulate metabolic activity. As thyroid levels and thermoregulatory behaviour have not previously been measured simultaneously in any mammalian species it is difficult to ascertain any real results from this observation however it does suggest the importance of behaviour in thermoregulation in marsupials. The use of autonomic effectors to regulate thermogenesis may be the basis of marsupial thermoregulation but the use of temperature-seeking behaviour is a major contributing factor to the homeostasis of marsupial body temperature. This will be further discussed in Chapter 6.

Another explanation for the differences observed in total thyroid hormone concentrations in colony and gradient animals may be related to the age of the animals used. This is supported by the observation that juvenile animals had consistently higher levels of plasma thyroid hormone concentrations than older animals of the same gender. The mean age of the adult male animals in the colony at the time of cardiac puncture was 714 days compared with 1006 days for the gradient animals although there was

much variance between individual ages between each group. According to Tomasi (1991) aging depresses thyroid activity and results in generally lower levels of metabolism typically seen in older animals. Thyroid hormone production is lowered with age in humans (Barzel, 1989) and aged rats show a reduced level of cerebral tissue responsiveness to thyroid hormones (Mooradian et al., 1998). The animals utilised in the thermal gradient in this study were older animals (generally past reproductive age) and thus it is highly likely that this had some influence on their general thyroid status. As a consequence, lower thyroid levels were noted in the gradient animals as thyroid hormone production is decreased as a consequence of the aging process. However, juvenile and adult *M.domestica* did not differ significantly with respect to levels of Tb regulation despite a lack of circadian control of Tb in juveniles (see Chapter 3). Therefore any loss of thyroid control was not influential in altering the Tb rhythm of the aged animals in the gradient. It is likely that these animals were able to seek certain Ta to modulate their Tb rhythms and account for any loss in autonomic thermoregulation due to depressed thyroid activity. It is also of interest to note that significant differences were observed between T_3 levels in juveniles and adults but not between adult and juvenile T_4 levels.

Gender has been identified as another influencing factor affecting thyroid hormone levels in other mammals and is seen to have some influence on total T_4 and total T_3 levels in this study of *M.domestica*. Females had significantly higher levels of total T_4 and total T_3 than males held in similar environmental conditions. This reflects a higher level of metabolism in females which is known to increase during reproduction (Thompson and Nicoll, 1986; Harder et al., 1996). Alternatively, the different levels of thyroid hormones in individual animals may reflect differences in binding protein levels.

According to Fukuhara et al., (1996), exposure to the cold activates the hypothalamo-pituitary-thyroid system in rats leading to increases in thyroid secretions. However there is no clear evidence which links

increased thyroid hormone metabolism on short-term cold exposure to changes in resting metabolism (Dauncey, 1990) and according to Mory et al., (1981) there is a limited role for thyroid hormones during cold exposure in rats. However, an increase in plasma TSH concentration followed by an increase in plasma T_3 and T_4 levels is a commonly observed reaction to cold exposure (Arancibia et al., 1996). The limited data obtained in this study from one male *M.domestica* at three different environmental temperatures illustrates that at lower ambient temperatures, T_3 and T_4 levels increase greatly. Similar observations have been observed in the marsupial, *Antechinus stuartii* (Withers and Hulbert, 1988) in which large increases in thyroid activity and metabolic rate were found following cold acclimation at a Ta of 5°C compared to a warm acclimation at a Ta of 25°C. This supports the theory that acute exposure to cold results in thyroid stimulation and an increase in the secretion of thyroid hormones in marsupials which can then be maintained at prolonged exposures to cold due to the absence of BAT (Hayward and Lisson, 1992) and noradrenaline-mediated NST in small marsupials (eg Dawson and Olson, 1988). According to Guernsey and Edelman, (1983) thyroid hormones play a significant role in the NST component of cold adaptation in mammals and Li et al., (2001) have recently shown that thyroid hormones contribute to cold adaptive thermogenesis in small mammals. Obviously, the limitations of the data in this study prevent this evidence from being conclusive but nevertheless highlight the importance of thyroid activity in thermoregulation in marsupials.

Thyroid hormones have been implied to have a significant role in the NST component of the adaptation to the cold (Guernsey and Edelman , 1983) although Triandafillou et al (1982) found that it is unlikely that thyroid hormones are mediators in BAT changes during cold acclimation in rats. A limited role of thyroid hormones as a mediator for BAT responses during cold adaptation has been proposed by Mory et al., (1981). The immediate response to cold exposure involves the secretion of TSH from the anterior portion of the pituitary gland (Reichlin et al., 1972) possibly involving the hypothalamus releasing TRH (eg Evans and Ingram, 1974). However, upon

initial exposure to the cold, Tb does not decrease and may even increase. Hypothermia only occurs in very severe cold exposure when the normal homeostatic thermoregulatory mechanisms fail (Dauncey, 1990). Laboratory-bred *M. domestica* does not thermoregulate well in the cold according to the evidence of this study yet thyroid hormones appear to increase to facilitate thermoregulation in the cold. Whether this is related to NST or some other physiological mechanism is unknown.

It is apparent that the thyroid gland has a role in marsupials thermoregulation, and increases its secretions in order to increase metabolism (and heat production) and consequently Tb. This is seen in both adult and juvenile *M. domestica* highlighting the importance of this endocrine gland in marsupial thermoregulation and mammalian thermoregulation generally. However, it is important to remember that the levels measured in this study were total plasma concentrations and therefore were affected by the concentrations of plasma thyroid-binding proteins and their affinity for T_4 and T_3. Obviously, other physiological adjustments also form the basis of a marsupial's ability to thermoregulate and behaviour may also play a role in thermogenesis in these unique mammals.

CHAPTER 6

BEHAVIOURAL SELECTION OF AMBIENT TEMPERATURES

6.1 Introduction

Behaviour is one of the fundamental mechanisms by which animals can regulate Tb irrespective of their autonomic mechanisms of thermoregulation. Behavioural thermoregulation has been shown to be a remarkably sensitive mechanism in many animals which functions to respond immediately to changes in Ta before major changes in internal Tb occur (Adair, 1976; Schmidt, 1978; Gordon, 1983b). This results in efficiencies in energy balance by reducing the occurrence of autonomic responses and compensates for insufficiencies of thermoregulatory mechanisms (such as panting) in the maintenance of thermal homeostasis (Schmidt, 1984).

Daily temperature rises observed in endotherms correspond to a regulated elevation in thermal set-point (Hensel, 1981) with daily fluctuations in Tb assumed to be the consequence of a daily fluctuation of the thermoregulatory set-point (Aschoff, 1970). Thermoregulatory behaviour is a valuable tool in the determination of whether observed changes in body temperature are due to a shift in this thermal set-point (Satinoff and Henderson, 1977). Such shifts are often used to explain regulated changes in Tb during hypoxia (Wood, 1991) or fever (eg Florez-Duquet et al., 2001). Indeed, one would expect that animals would tend to select higher Tas when their Tb is elevated and lower Tas when their Tb is decreased if the thermal set-point is shifted.

The use of behaviour to control core Tb is well documented for ectotherms as this is the main means of thermoregulatory control in these animals (eg. Sievert and Paulissen, 1996). In endotherms, the selection of

preferred ambient conditions has also been investigated, particularly in rodents. Some rodents appear to select ambient temperatures over a circadian cycle that follow a similar pattern to the circadian phases of core Tb (eg Scales and Kluger, 1987) while others select temperatures which are 180° out of phase with core Tb cycles (eg Refinetti, 1995b; Refinetti, 1996). In many cases, rodents select Tas that closely reflect their metabolic TNZ (Gordon, 1993b). Similarly, birds tend to select Tas that are within or just below the TNZ (Budgell, 1971; Laudenslager and Hammel, 1977).

The ability to behaviourally select preferred Tas is present at birth in small mammals such as rats and mice (Ogilvie and Stinson, 1966; Eedy and Ogilvie, 1970; Kleitman and Satinoff, 1982) although the importance of this behaviour in thermoregulation somewhat diminishes as physiological mechanisms mature (Leonard, 1974). This reduced need for behaviour may be a characteristic of laboratory-bred animals rather than animals in their natural habitats. As shown by Hofmeyr and Louw (1987) free-ranging springbok (*Antidorcas marsupialis*) rely less on autonomic thermoregulation when they have access to behavioural thermoregulation. The environment has been shown to play an important role in adaptive thermogenesis, in many free-living species including humans (eg Shido et al., 2001). Restrained animals and animals kept in artificial environments are deprived of normal thermoregulatory behaviour including interactions with other animals and the use of varying environmental conditions.

In adult mammals, thermoregulatory behaviour is utilised particularly during heat and cold stress (Stinson and Fisher, 1953; Gordon, 1983a; Schmidt, 1984; Morimoto et al., 1986) although this utilisation during exposure to these stressors becomes impaired with age (eg Owen et al., 1991). The selected Ta of mammals has also been shown to be unaffected by age; this has been shown in humans (Collins et al., 1981) and rats (Jakubczak, 1966; Owen et al., 1991). According to Sugimoto et al., (1999) behavioural thermoregulatory function in rats can also be altered by heat acclimation although this does depend on the method of heat exposure.

Behaviour, therefore, appears to be utilised to some degree by endotherms of all ages especially while physiological mechanisms are unable to maintain normal Tb effectively.

The role of behaviour in thermoregulation in marsupials has seldom been determined with respect to Selected Ta. Song et al., (1998) reported that *S. macroura* select high Ta near the TNZ during both torpor and normothermia to reduce energy costs. In this study, Selected Ta was determined in laboratory-bred *M. domestica* using a thermal gradient while simultaneously measuring core Tb. It was hypothesised that *M.domestica* would select Tas in a circadian pattern which closely reflected their circadian patterns of Tb. This would support the theory that behaviour is a component of the proposed set-point theory of mammalian thermoregulation.

6.2 Results

Recordings of continuous measurements of selected Ta for each *M. domestica* are given in Appendix 5. Recordings of selected Ta were significantly different between different animals ($F=1750.1$; $p<0.05$). A typical three day recording from a *M. domestica* after 24 hours to allow the animal to acclimatise to the thermal gradient is shown in Figure 6.2.1.

The mean daily selected Ta and core Tb was determined for each *M. domestica* as shown in Table 6.2.1. Interestingly, MOAG18 which had the highest mean core Tb selected a lower mean Ta and MOAG15 and MOAG17 with the lowest core Tbs had the higher mean selected Tas.

Figure 6.2.1 Typical Continuous Recording of Preferred Ambient Temperature (Ta) of an adult M. domestica (MOAG13) while in a longitudinal thermal gradient
[one day of recording is shown; recordings made every 6 seconds]

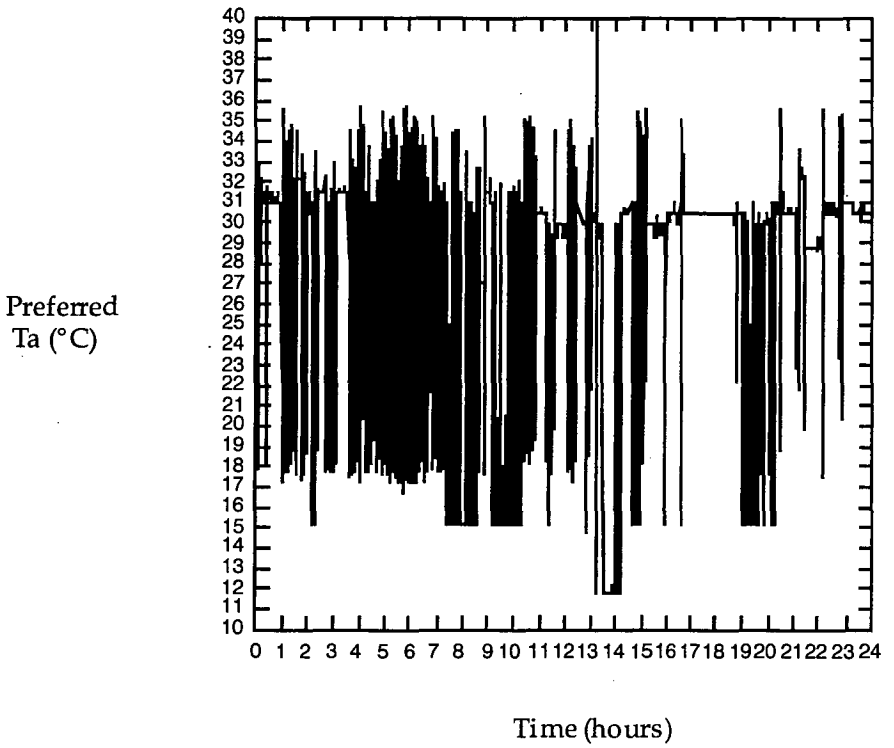


Table 6.2.1 Mean Core Body Temperature (Tb) and Selected Ambient Temperature (Ta) of *M. domestica* while in a Thermal Gradient for three days

Animal	Core Tb (°C) (X±SD)	Selected Ta (°C) (X±SD)
MOAG13	34.5±0.5	29.7±3.8
MOAG15	34.3±0.6	30.4±2.3
MOAG16	34.6±0.4	29.2±2.6
MOAG17	34.3±0.6	31.4±3.1
MOAG18	34.8±0.6	28.7±3.7

A histogram of selected Ta versus number of observations for all five *M. domestica* shows a distribution with a slightly positive skew and a mean preference of 29.9±1.1°C (Figure 6.2.2). The number of observations at each Ta for each *M. domestica* are shown in Figures 6.2.3 (a-e).

Figure 6.2.2 Mean frequency of responses to preferred ambient temperature in *M. domestica* (n=5)

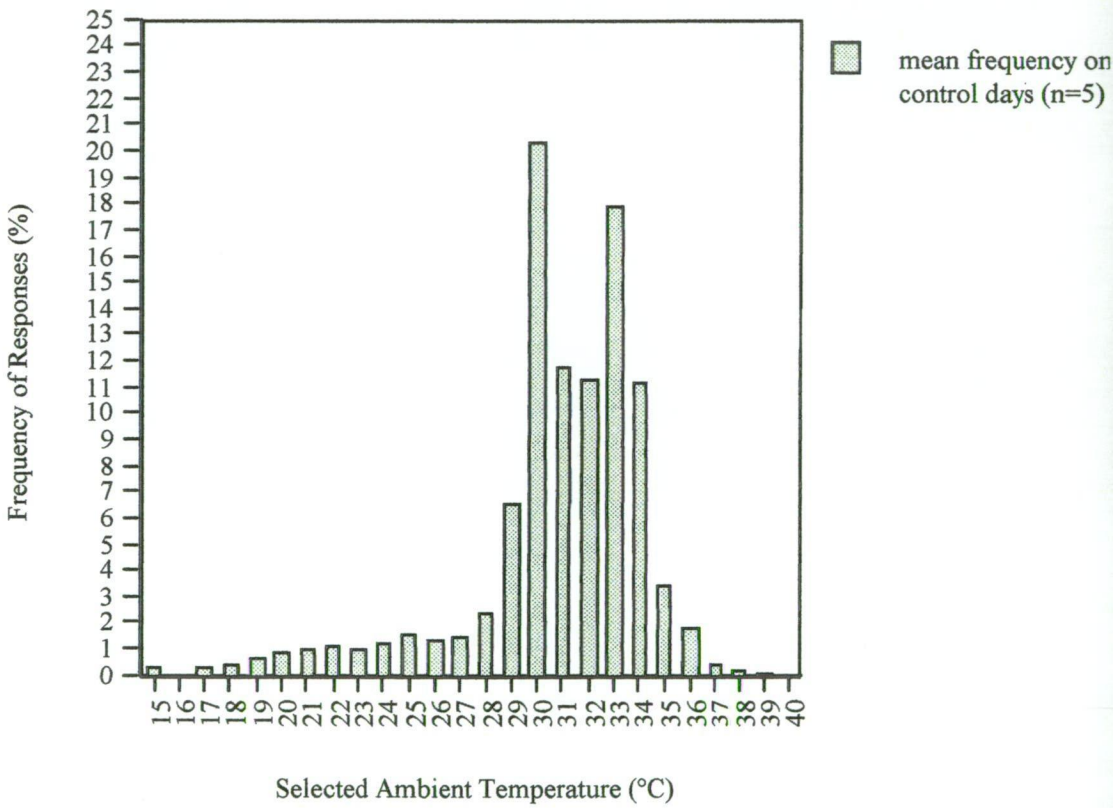


Figure 6.2.3a Frequency of responses to selected ambient temperature in MOAG13

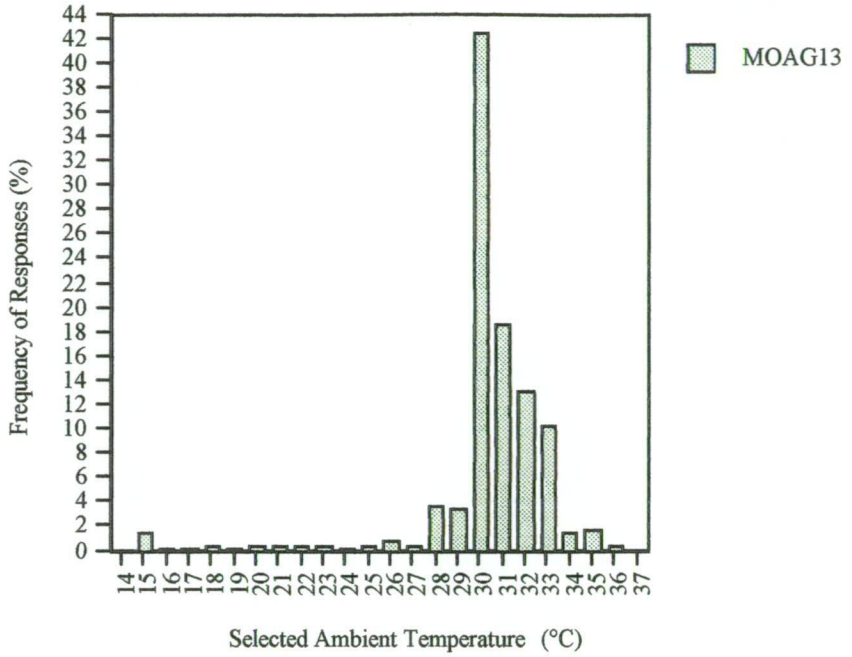


Figure 6.2.3b Frequency of responses to selected ambient temperature in MOAG15

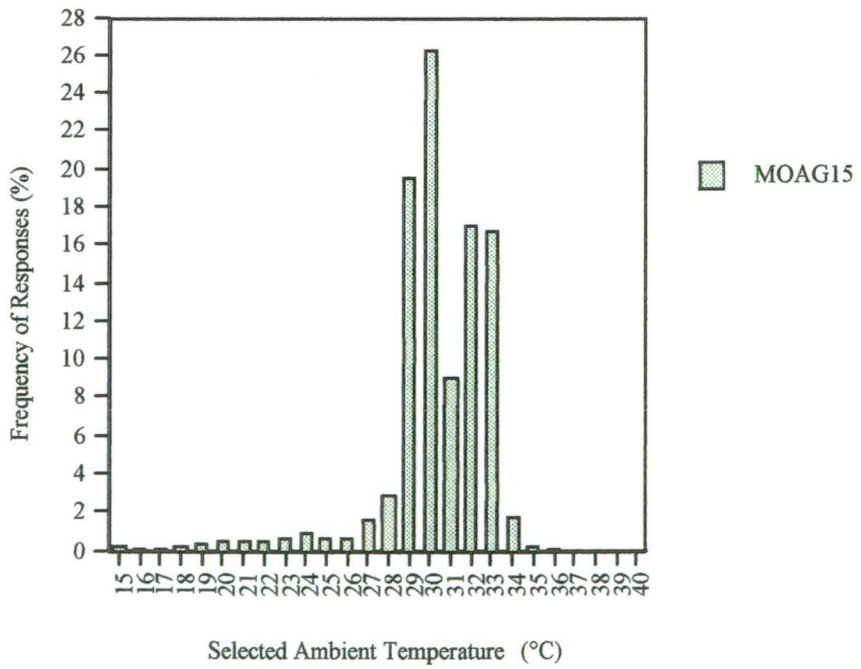


Figure 6.2.3c Frequency of responses to selected ambient temperature in MOAG16

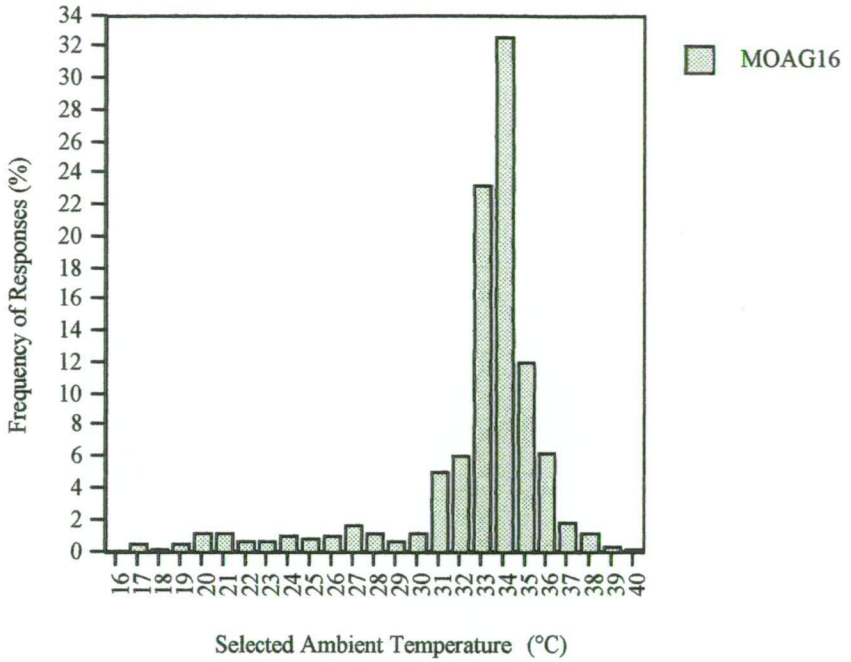


Figure 6.2.3d Frequency of responses to selected ambient temperature in MOAG17

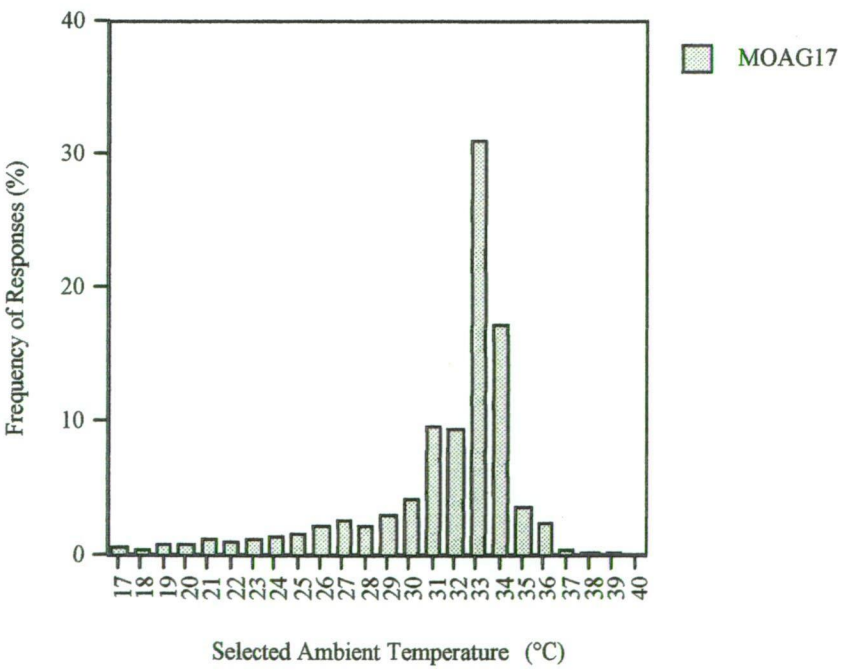
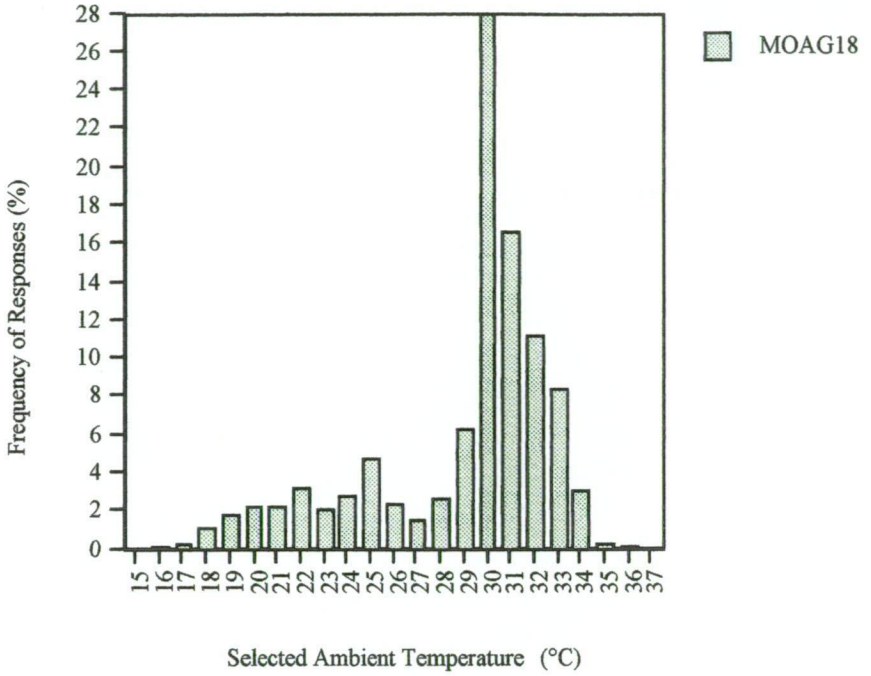


Figure 6.2.3e Frequency of responses to selected ambient temperature in MOAG18



Fourier analysis of all recordings for each animal indicated the lack of a distinct circadian rhythm of selected Ta (see Appendix 10 for examples of analysis plots). Data were analysed using a single series Fourier analysis and results plotted by period using transformations in which the mean was subtracted and detrended. Table 6.2.2 shows the amplitude of selected Ta, acrophase (timing of peak), and period for selected Ta in each *M. domestica*. A period of 24 hours was determined for selected Ta in MOAG16 and MOAG18 although this was not as well defined as the 24-hour rhythm of Tb seen in these animals. In addition, MOAG18 also exhibited strong periods at 7, 14, 18 and 36 hours. The periods for each Fourier analysis of selected Ta can be found in Appendix 10.

Table 6.2.2 Analysis of Selected Ta: Amplitude, acrophase (timing of peak), and period of selected Ta in *M. domestica*

[period was calculated by Fourier analysis; amplitude is the difference between the maximum and minimum Ta selected; acrophase is given in 24-hour time]

Animal	Amplitude (°C)	Acrophase	Period (hours)
MOAG13	21.5	04:58	48
MOAG15	19.0	05:35	36
MOAG16	20.5	02:09	24
MOAG17	21.4	21:56	36
MOAG18	20.3	16:53	24

6.3 Discussion

Thermoregulation in mammals is a complex control process involving many effector mechanisms. Each effector involved in the control of core Tb is influenced by the Ta. However, many studies of thermoregulatory control in mammals have failed to address the influence of Ta on thermogenic mechanisms instead concentrating mainly on physiological effectors and their roles. Selected Tas have been investigated in a number of endotherms and ectotherms but often the experimental evidence has been limiting due to the short duration of the observations of animals in thermal gradients. As Tb alters over a circadian cycle of about 24 hours in most animals, selected Ta may also be subject to such variations and cycles. Rhythms of temperature selection have been observed in various mammals particularly rodents (eg Gordon, 1994), but the physiological significance of these rhythms is yet to be determined.

According to this study, *M. domestica* do select various Tas throughout a 24 hour period as previously observed in the rat (Refinetti and Horvath, 1989). However, no distinct rhythm of Ta preference is clearly

recognised in *M. domestica*. There was a tendency for all *M. domestica* to spend the majority of their time in the centre of the gradient rather than at the extreme ends of heat or cold. Food was placed in this central area of the gradient which may attribute to this finding however animals were randomly observed in the gradient during the daylight hours and were often found to be sleeping in the central regions of the gradient and occasionally favouring one particular end. This form of behaviour has also been observed in deer mice (Stinson and Fisher, 1953). Unlike deer mice, however, *M. domestica* do spend some time at the extreme ends of the thermal gradient particularly when they are most active during the evening hours. This was also observed in the *P. breviceps*. A pilot experiment of the study involved placing food at either end or in the middle of the gradient and no changes in behaviour were evident. It was found that animals had the best access to all regions of the gradient by placing the food in the centre and so this was the position chosen for all experiments.

The lack of rhythmicity in temperature selection generally observed in *M. domestica* is not totally unusual as not all eutherians have been shown to display rhythms of temperature preference. Evidence of daily rhythms of temperature selection have previously been found in rodents such as rats (Briese, 1985; Gordon, 1994), hamsters (Gordon et al., 1986; Refinetti, 1995a; Refinetti, 1995b) and the Chilean degus (Refinetti. 1996) but not in ground squirrels (Refinetti, 1995a). It was suggested by Refinetti (1995a) that this lack of Ta rhythm in the ground squirrel was related to its diurnal activity and that in contrast, nocturnal endotherms do exhibit a robust daily rhythm of Selected Ta which is 180° out of phase with their rhythms of core Tb. This does not explain observations from this study.

M. domestica is a nocturnal marsupial and was expected to show a distinct rhythm of selected Ta as seen in nocturnal eutherians. The absence of such a pronounced rhythm in *M. domestica* may be attributed to the fact that the animals were bred and maintained in a colony at a relatively constant Ta. This will have altered the ability of the animal to utilise

environmental conditions for thermal homeostasis as part of thermoregulatory development from a young age. Huddling contributes significantly to thermoregulation in small marsupial species such as *P. breviceps* (Fleming, 1980). Whether such a behavioural strategy is utilised by *M. domestica* is not reported in the literature, although likely due to the small size of the species and the fact that the young develop in a nest where huddling while they are newborn ectotherms would be of great benefit to Tb control.

The use of Ta in marsupial thermoregulation cannot be disregarded as a mechanism in overall thermoregulation despite the less than convincing results of this study. Many rhythms of Ta selection previously reported in rodents are out of phase with the simultaneous rhythms of Tb indicating that Ta may oppose rather than modulate core Tb (eg Refinetti, 1995b). This has also been observed in male human subjects (Shoemaker and Refinetti, 1996). This out of phase pattern was not observed in *M. domestica* but as the animals did not just select the Ta that they were accustomed to in the colony, the study does suggest that the availability of a range of Ta did have some impact on the animal and possibly its thermogenesis.

According to Smith and Fisher, (1956), a thermal gradient of a constant temperature can result in restlessness with varying degrees of activity observed. Confinement within the thermal gradient may have caused restlessness in the animals studied although they were sourced from a colony where they were maintained in confined enclosures at a relatively constant room temperature. It would have been of interest to study the animals in the thermal gradient when it was set at a constant temperature throughout. Observations from a single *P. breviceps* in our laboratory have suggested that restlessness is a common occurrence when an animal is placed in a constant temperature gradient. This may be an indication of a reduction of thermal comfort in this animal especially as huddling is a common thermoregulatory strategy used by *P. breviceps*.

The range of Ta selected by endotherms is quite variable. The selected Ta range of birds is within or just below the TNZ (Budgell, 1971; Laudenslager and Hammel, 1977). Rats select Tas as low as 19°C (Gordon, 1988; Refinetti and Horvath, 1989) and as high as 32°C (Refinetti and Carlisle, 1986a), guinea pigs have been found to select 30.6°C (Gordon, 1986) and mice 30.9°C (Gordon, 1985). Differences in selected Ta exist between different strains and species, which are either real or attributable to acclimation. Refinetti and Horvath (1989) indicated that intrasubject variability of selected Ta in rats was significantly lower than intersubject variability. Thus, animals of the same species may show marked differences in selected Ta while remaining relatively consistent with respect to their own individual preferences. This is seen in marsupials with some consistencies in data from days of individual animals but obvious differences observed intraspecifically between individual *M.domestica* where mean selected Ta ranges from 29.2°C to 31.4°C. Unlike autonomic functions behaviour is therefore a highly individual-specific parameter of thermoregulation.

Results from rats conflict with those of other endotherms with respect to thermoregulatory behaviour. According to Gordon (1993b), guinea pigs select Tas near the upper end of the TNZ, hamsters, mice and gerbils select Ta values near the lower or middle end of the TNZ, but rats select Ta outside of their TNZ. The preference for a Ta range below the TNZ has been observed in rats (Poole and Stephenson, 1977; Gordon, 1987) and rats have also been shown to select a Ta within their TNZ (Hart, 1971). It has been suggested by Gordon et al., (1991) that a rat's initial preference for a cooler Ta is stress-related as after a few hours in a thermal gradient, it begins to select warmer temperatures. Such stress-related results was not apparent in this study as animals were allowed to acclimatise to the gradient for at least 24 hours before recordings were analysed. The TNZ of *M. domestica* is 28-35°C (Dawson and Olson, 1988; Harder et al., 1996). *M. domestica* tend to select Ta just below or at the lower end of the TNZ and there is no indication that they select warmer temperatures as they spend longer in the gradient.

M. domestica did not exhibit circadian patterns of Ta selection in phase with Tb as observed in humans (Cabanac et al., 1976) or 180° out of phase with Tb as observed in many rodents (eg Briese, 1985) and humans (Shoemaker and Refinetti, 1996). This is despite the observation that a variety of Ta are selected by these marsupials throughout a 24-hour period. According to Refinetti and Carlisle (1986b) temperature changes in the anterior hypothalamus affect both behavioural and autonomic thermoregulation whereas temperature changes in the posterior hypothalamus affect only behavioural thermoregulation. This has also been suggested by Satinoff and Rutstein (1970) who observed an incapacity for autonomic thermoregulation in rats with anterior hypothalamic lesions however the animals were still able to maintain core Tb through behavioural means. If core Tb is controlled by a hypothalamic set-point in *M. domestica* then behaviour does not act as a major influencing factor however it may be utilised when autonomic effectors are unable to effectively maintain Tb. Furthermore, the lack of distinct circadian patterns of Ta selection in relation to circadian patterns of Tb in this species demonstrates a possible minor role of Ta in normal marsupial thermogenesis. Future studies looking at Ta selection in marsupials with anterior and/or posterior hypothalamic lesions may provide some insight into the role of the thermoregulatory centres in behavioural selection of Ta.

It is apparent that unlike many other homeostatic systems the endothermic thermoregulatory system does rely on behaviour to some degree to modulate its regulation. Thermoregulatory control centres are highly sensitive and receive continual input from changes in skin and core temperatures resulting in the production of effective responses by both autonomic and behavioural effectors (Hensel, 1973). Grooming, huddling and postural changes, as well as actively seeking preferred ambient temperatures, are all behaviours related to thermoregulation and have been observed in many animals including marsupials (eg Fleming, 1980). According to Gordon (1983a), actively selecting a preferred Ta is favourable

as it is less energy-demanding and more efficient in minimising a thermal load than autonomic thermoregulation. In fact, many researchers agree that due to its lower energy requirements behavioural thermoregulation is the preferred effector mechanism for homeostatic temperature regulation in birds and mammals (see Gordon, 1994).

This study has shown that *M. domestica* can utilise the environment to behaviourally select a Ta although a regular pattern of selection may not always be apparent. Whether this use of behaviour in thermoregulation helps minimise thermal loading in these animals is not apparent from this study. Simultaneous measurements of various thermoregulatory effectors including piloerection, sweating, and vasomotor control of vasculature need to also be considered to determine this.

The laboratory-bred *M. domestica* used in this study are not a true representative of native *M. domestica* as they have been bred and maintained under relatively constant laboratory conditions. This makes them a less than ideal animal to use to investigate behavioural thermoregulation. Exposing these animals to a variable Ta environment was a novel experience to them. Exposure of the colony of *M. domestica* to a cycle of variable Tas and then checking their Selected Ta would have been of interest. Unfortunately this was not possible due to quarantine restrictions on experimental rooms. According to Geiser and Ferguson (2001) captive-bred feathertail gliders (*Acrobates pygmaeus*) exhibit poor expression of torpor and thermal performance compared to their field counterparts. This highlights the importance of using wild rather than laboratory animals in thermoregulatory studies.

As stated by Smith and Fisher, (1956), behavioural selection of Ta can be regarded as a very simple and generalised characteristic of animals. Temperature selection as a means of regulating core Tb is of paramount importance to ectotherms which usually lack physiological means of fine tuning their Tb. From an evolutionary point of view it would seem unwise

to assume that endotherms have totally eliminated this thermoregulatory behaviour to rely totally on internal homeostatic mechanisms. Many physiological effectors of thermoregulation can be energy demanding. On the other hand, behavioural responses tend to be quicker and less costly in energetics. This suggests that it would be more beneficial for endotherms to use a combination of behaviour and physiology to efficiently thermoregulate. This is apparent from studies reporting alterations in both autonomic and behavioural thermoregulation in starved animals (eg Yoda et al., 2000). Although this may not always be seen in normal thermoregulation this combination may be of importance when faced with conditions which result in moderate or pronounced degrees of hypothermia or hyperthermia. Assessing the ability of an animal to utilise its environment when faced with such a temperature altering stressor may contribute to our understanding as to the importance of behaviour in overall endothermic thermoregulation.

Overall, the biological significance of thermoregulatory behaviour in endotherms such as marsupials is still far from clearly understood. Any future behavioural studies on thermoregulation in marsupials should involve animals obtained and maintained under natural habitat conditions and should incorporate field studies where possible. The importance of this is also highlighted in recent studies (eg Geiser and Fergusin, 2001). Such field studies will determine the role of behaviour in the natural Tb control in marsupials.

CHAPTER 7

THERMOREGULATORY RESPONSES TO BACTERIAL ENDOTOXIN (LIPOPOLYSACCHARIDE)

7.1 Introduction

It is well established that pathogenic material (bacterial or viral) will generally induce a hyperthermic response in mammalian species when administered intravenously (eg Hellon, 1975). Thermoregulatory responses to bacterial endotoxin or lipopolysaccharide (LPS) however, are varied among eutherian mammals with monophasic and biphasic rises in Tb observed (eg Stitt et al., 1985; Kozak et al., 1994). The general consensus that a febrile response (ie increased Tb) will be observed in response to bacterial LPS has been challenged by investigations indicating a drop in Tb in response to LPS in small mammals (eg Connor and Krass, 1961; Feldberg and Saxena, 1975). This non-febrile response has been proposed to be related to a larger surface area-to-volume ratio resulting in an increase in overall heat loss (Feldberg and Saxena, 1975). It is possible, however, that the failure to observe febrile responses to endotoxin or lipopolysaccharide may be due to the lengthy time course required of these fevers (Kozak et al., 1994) or may be related to dosage as lethal dosages of pyrogens lead to hypothermia as a consequence of shock (Romanovsky et al., 1996).

Many studies have involved measuring Tb during fever at short intervals (eg Stitt et al., 1985) but to achieve a true observation of the effects of bacterial endotoxin or any pyrogen on core Tb, continual measurements are necessary due to the cyclical variations of mammalian Tb. This has been demonstrated by Severinsen and Oritsland, (1991) who observed a prolonged febrile response in rats using temperature sensitive transmitters. In addition, normal circadian variations in thermoregulation may moderate or augment hyperthermic febrile responses particularly if both are

controlled by mechanisms involving the hypothalamic set-point. This further highlights the need for continuous measurements of Tb in studies of fever and thermoregulation.

Behavioural responses to bacterial endotoxin and other pyrogens have been well documented in ectothermic thermoregulation (eg Hutchison and Erskine, 1981; Boorstein and Ewald, 1987; Hallman et al., 1990). Typically, when exposed to pyrogens, ectotherms select warmer temperatures in their environment resulting in an increase in core Tb. However, not all ectotherms can develop behavioural fevers (eg Laburn et al., 1981; Marx et al., 1984). Thermoregulatory behaviour during fever is not as well documented in mammalian species although existing studies do indicate that behaviour may play a role (eg Crawshaw and Stitt, 1975). While febrile, endotherms are observed to seek warmer environments (eg Florez-Duquet et al., 2001) indicating that an adjustment in the thermoregulatory set-point occurs in response to pyrogens. As behaviour may play a role in fever, it is possible that ectothermic and endothermic fever share a common phylogenetic origin as suggested by Myhre et al., (1977). However, further studies of more diverse animal species (ectothermic and endothermic) need to be made to determine the importance of thermoregulatory behaviour in the endothermic fever response.

Thermoregulatory responses to bacterial LPS (from *E. coli*) in *M. domestica* were investigated in this study. Results from a single *P. breviceps* exposed to *E. coli* are also included for comparison. Changes in core Tb (via temperature-sensitive transmitters) and selected Ta (via a thermal gradient) in response to injections (i.m.) of LPS were determined for both marsupial species. Sensitivity to repeated i.m. injections of LPS (over a five week period, on a weekly basis) was also determined for one animal. It was hypothesised that these marsupials would exhibit a significant monophasic rise in Tb in response to LPS and that warm-seeking behaviour would be observed while febrile.

7.2 Results

Appendix 6 illustrates the continuous measurements of core Tb and selected Ta in each animal during saline and LPS conditions. Different patterns of Tb were observed in each animal although all animals experienced an increase in core Tb in response to the bacterial endotoxin (ie. LPS). Selected Ta was also affected by LPS although effects varied greatly among individuals and the impact of LPS on changes in Ta selection was minimal in *M. domestica*. LPS had a greater effect on Ta selection in the one *P. breviceps* with warmer Tas selected by this animal while febrile.

One animal (MOAG13) received weekly injections of LPS paired with saline control injections for a period of five weeks. Increases in Tb were noted with each weekly injection of LPS however the magnitude and pattern of the increase did differ between subsequent treatment weeks.

7.2a Effects of Saline on Core Body Temperature and Selected Ambient Temperature

The daily mean core Tb and mean selected Ta of *M.domestica* (taken from continuous measurements for 24 hours post-injection) were 34.4±0.2°C and 30.91± 1.5°C respectively (see Table 7.2.1a and Table 7.2.1b). Tables 7.2.1a and 7.2.1b also show the mean core Tb and mean selected Ta for each *M. domestica* over a 24 hour period under control and saline conditions.

In *M. domestica*, saline did not have a significant effect on Tb when compared to normal control values (Two factor ANOVA: $p>0.05$; $F=4.0$). Similarly, no significant difference was observed between mean values calculated for individual *M. domestica* (ANOVA: $p>0.05$; $F=1.9$). However, when comparing 15 minute means of Tb a significant difference between saline injection and control Tb was noted in three *M. domestica* (t-test: MOAG13; $p<0.001$; $t=5.6$; MOAG17; $p<0.001$; $t=-5.8$; MOAG18; $p<0.001$; $t=4.3$). Mean differences in Tb (ΔTb) ranged from 0.1°C to 0.2°C in individual animals as shown in Table 7.2.1a.

With respect to the selection of Ta, no significant difference was noted between control and saline injection in *M. domestica* (ANOVA: $p>0.05$; $F=3.0$), however, significant differences existed between individuals (ANOVA: $p<0.01$; $F=38.6$). Similarly to Tb, saline did have a significant effect on selected Ta when comparing individual measurements of Ta selection in two *M. domestica* (t-test: MOAG15; $p<0.001$; $t=5.2$; MOAG16; $p<0.001$; $t=6.6$). Interestingly, individuals which showed significant differences in Ta selection were not the same individuals that showed significant differences in core Tb. Mean differences in Ta selected (ΔTa) ranged from 0.2°C to 1.1°C in individual animals as illustrated in Table 7.2.1b.

Table 7.2.1a Mean Core Body Temperature (Tb) of *Monodelphis domestica* in response to intramuscular injections of Isotonic Saline

Individual animals were injected with saline (1mg/kg).
ΔTb represents mean difference in Tb.
[mean±SD was calculated from measurements taken over a 24 hour period for saline and over a period of three days under control conditions for control]

Animal	Control Tb (°C)	Saline Tb (°C)	ΔTb (°C)
MOAG13	34.5±0.5	34.2±0.7	0.2
MOAG15	34.3±0.6	34.2±0.7	0.1
MOAG16	34.6±0.4	34.43±3.6	0.1
MOAG17	34.3±0.6	34.5±0.5	0.2
MOAG18	34.8±0.6	34.6±0.6	0.2
ALL	*34.5±0.2*	*34.4±0.2*	*0.2*

Table 7.2.1b Mean Selected Ambient Temperature of *Monodelphis domestica* in response to intramuscular injections of Isotonic Saline

Individual animals were injected with saline (1mg/kg).
ΔTa represents mean difference in Ta.
[mean±SD was calculated from measurements taken over a 24 hour period for saline and over a period of up to three days under control conditions for control]

Animal	Control Ta (°C)	Saline Ta (°C)	ΔTa (°C)
MOAG13	30.3±2.7	30.2±2.3	0.2
MOAG15	33.8±1.8	33.0±1.6	0.8
MOAG16	32.4±1.9	31.3±2.1	1.1
MOAG17	30.8±3.2	31.1±2.8	0.3
MOAG18	29.2±2.3	28.9±2.4	0.3
ALL	*31.3±1.8*	*30.9±1.5*	*0.5*

7.2b Effects of Bacterial Endotoxin on Core Body Temperature

Core Tb and selected Ta values for two *Monodelphis domestica* exposed to paired saline injections (ie saline [A] followed by saline [B] 24 hours later) are illustrated in Table 7.2.2. In MOAG13, mean core Tb decreased by 0.5°C with the second saline injection and mean selected Ta increased by 4.5°C. Similarly, in MOAG16 the second saline injection reduced mean core Tb by 0.1°C but mean selected Ta was also decreased by 0.3°C. Overall there was no significant difference in mean Tb resulting from the two consecutive saline injections (ANOVA: $p>0.05$; $F=0.8$). However, MOAG13 did show significant differences between core Tb during the two saline conditions (t-test; $p<0.001$; $t=7.7$). Similarly significant differences between selected Ta during the two saline conditions were also observed in MOAG13 (t-test; $p<0.001$; $t=3.1$). This animal was noted to be quite agitated on the first saline treatment day which may account for this significance. The frequency of responses to Ta are illustrated in Figures 7.2.1 (a-b) for each saline treatment for MOAG13 and MOAG15 respectively.

Table 7.2.2 Core Body Temperature (Tb) and Selected Ambient Temperature (Ta) of two *Monodelphis domestica* in response to two consecutive intramuscular injections of Isotonic Saline

Saline A represents initial injection of saline (1mg/kg), saline B represents a second injection of saline (1mg/kg) 24 hours later
[mean±SD was calculated from measurements taken over a 24 hour period from the time of injection]

Animal	Saline [A] Tb (°C)	Saline [B]Tb (°C)	Saline [A] Ta (°C)	Saline [B] Ta (°C)
MOAG13	34.7±0.4	34.2±0.5	29.4±4.2	33.9±2.3
MOAG16	34.2±0.8	34.1±0.6	33.3±1.8	33.1±1.4
ALL	*34.5±0.4*	*34.2±0.1*	*31.4±2.8*	*33.5±0.6*

Figure 7.2.1a The effect of two successive saline injections on the frequency of responses to ambient temperature in MOAG13

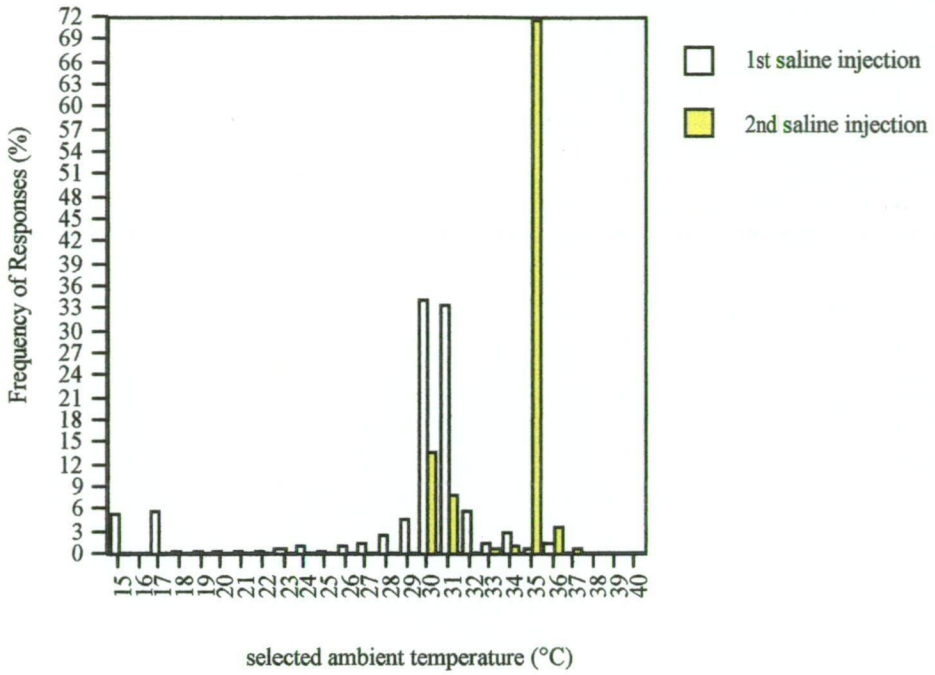
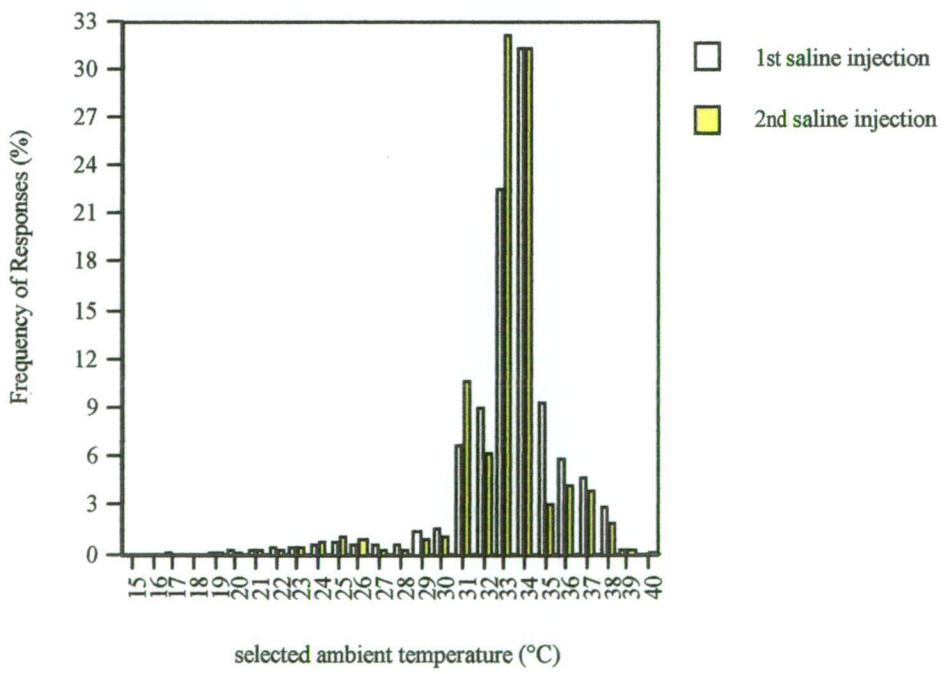
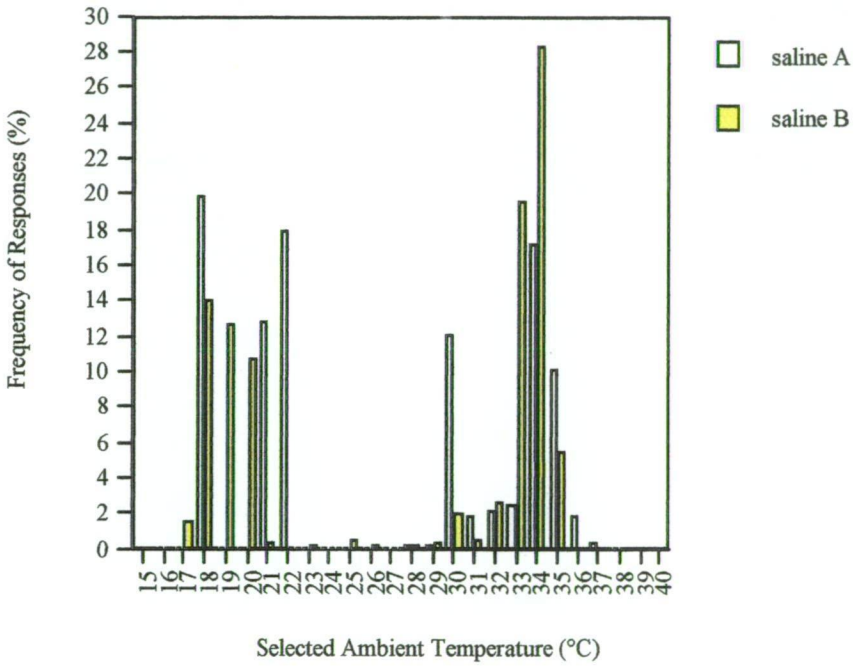


Figure 7.2.1b The effect of two successive saline injections on the frequency of responses to ambient temperature in MOAG15



Similarly, Selected Ta in the P. breviceps was significantly different between the two saline treatments (t-test; $p<0.001$; $t=-13.1$). The frequency of responses to Ta are shown in Figure 7.2.1c for this animal.

Figure 7.2.1c The effect of two successive saline injections on the frequency of responses to ambient temperature in PETAG1



An increase in core Tb was noted in each of the five *M. domestica* after the injection of *E. coli* LPS in comparison to the control (saline). The lag period between injection time and a noted increase in core Tb ranged from 1.5 hours to 4.5 hours with an average latent period of 3 hours observed. The increase in core Tb was observed for at least 3.5 hours to a maximum of 9.75 hours with an average of 5.8 hours. Mean core Tb was calculated for each animal during this affected time and increases ranging from 0.4-1.3°C were noted. Mean Tbs observed during control and "febrile" conditions (calculated from the time period affected by endotoxin) are illustrated in Table 7.2.3. A similar result was observed in PETAG1 with a latent period of 108mins and an increase in mean core Tb of 0.9°C for a period of 5.75 hours.

Table 7.2.3 Mean Core Body Temperature (Tb) of *M. domestica* in response to intramuscular injections of Isotonic Saline or Bacterial Endotoxin (*E.coli* LPS).

Latent period refers to elapsed time before endotoxin affected and time affected refers to the length of time the endotoxin increased the core Tb; ΔTb represents difference between Tb measured during saline period and Tb measured during period affected by LPS; all increases in core Tb due to LPS were significant

Animal	Condition	Latent period (mins)	Time affected (hours)	Core Tb (°C) (X±SD)	ΔTb (°C)
MOAG13	saline	-	-	33.6±0.4	-
MOAG13	*E.coli* LPS	85	6.5	34.9±0.4	1.2
MOAG15	saline	-	-	33.4±0.3	-
MOAG15	*E.coli* LPS	191	3.5	34.6±0.4	1.3
MOAG16	saline	-	-	34.2±0.2	-
MOAG16	*E.coli* LPS	273	4.75	35.0±0.2	0.8
MOAG17	saline	-	-	34.1±0.3	-
MOAG17	*E.coli* LPS	75	3.0	34.8±0.2	0.7
MOAG18	saline	-	-	34.3±0.2	-
MOAG18	*E.coli* LPS	185	9.75	34.7±0.3	0.4
ALL	*saline*	-	-	*33.9±0.4*	-
ALL	*E.coli LPS*	*161.8*	*5.8*	*34.8±0.2*	*0.9*

As the endotoxin increased core Tb for less than 24 hours, circadian effects of bacterial endotoxin on Tb are not clear from the results obtained. Circadian patterns of core Tb recorded from individual *M. domestica* while subjected to "febrile" conditions are presented in Appendix 6 with a typical recording shown in Figure 7.2.2a. Time periods where Tb was increased as a result from LPS injection were statistically analysed to determine significant changes in Tb as a reflection of circadian phase. A significant increase in mean Tb due to LPS injection was noted (ANOVA: $p<0.01$; $F=26.8$) with no significant difference between responses observed in individuals (ANOVA: $p>0.05$; $F=1.8$). Results obtained from individual *M. domestica* also indicate significant differences ($p<0.01$) between saline and endotoxin Tbs over the time phase in which an endotoxin-induced increase in Tb is noted. Similarly, the *P. breviceps* showed a significant increase in core Tb during the time at which endotoxin elevated Tb above that observed

during the saline control (t-test: $p<0.001$; $t=35.3$). This is illustrated in Figure 7.2.2b.

Figure 7.2.2a Continuous Body Temperature (Tb) Recordings from a *M. domestica* (MOAG13) during a four day period - the effects of saline and *E. coli* LPS injections

[saline was injected at 0930 hours on day 2;
LPS was injected at 0935 hours on day 3]

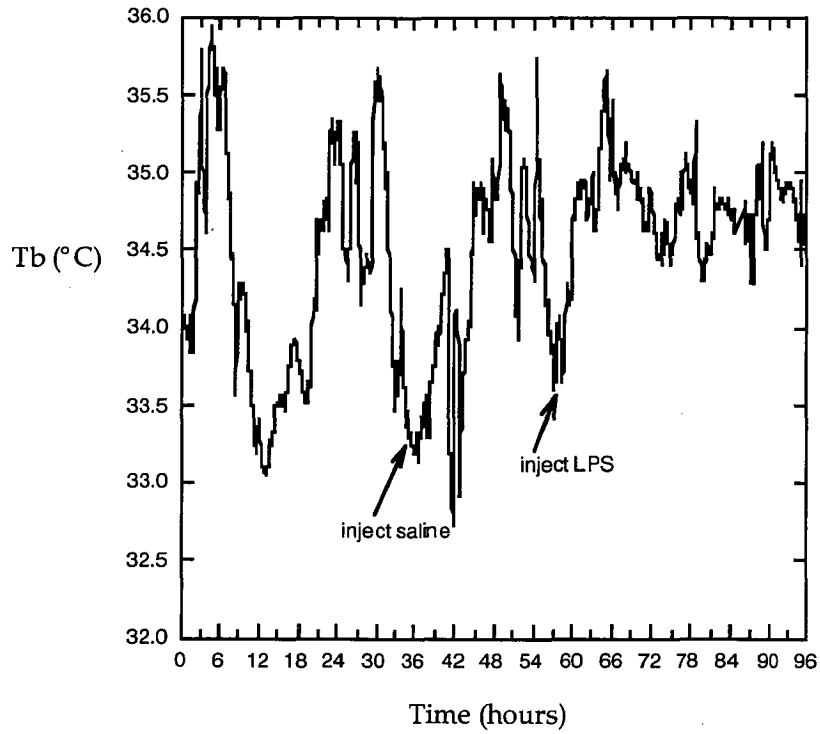
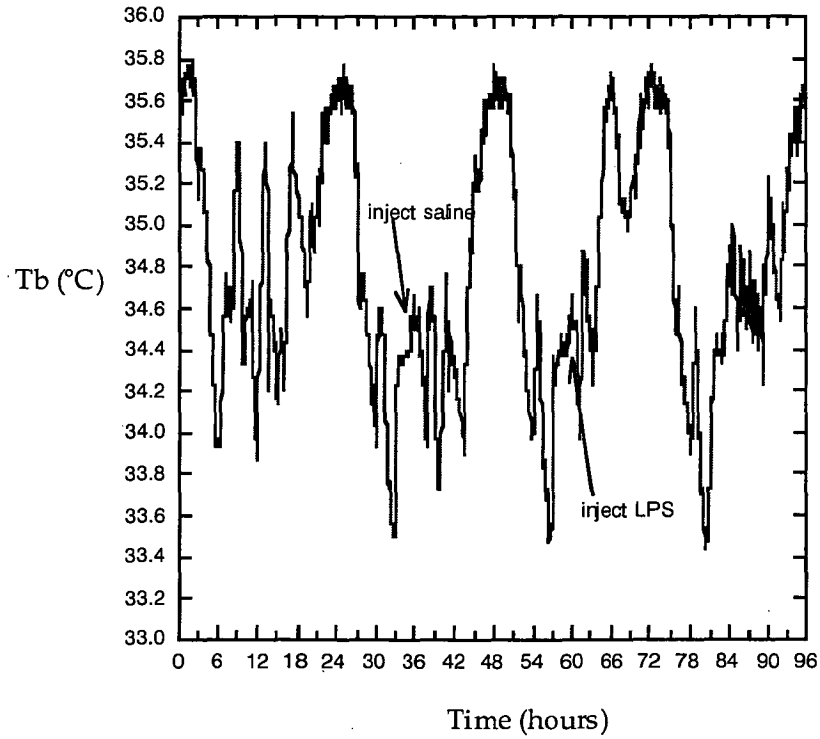


Figure 7.2.2b Continuous Body Temperature (Tb) Recordings from one *P. breviceps* - the effects of saline and *E. coli* LPS injections
[saline was injected at 1340 hours on day 2;
LPS was injected at 1315 hours on day 3]



Direct comparisons were also made between Tb measurements from three *M.domestica* which experienced an endotoxin-induced increase in Tb in a common time phase (ie 14:03 to 17:29). A highly significant increase in endotoxin-induced Tb was noted during this time phase (ANOVA: $p<0.01$; $F=181.3$).

The effect of LPS on normal circadian rhythms of Tb was determined in each animal using Fourier analysis. As the LPS caused an increase in Tb for a period for (on average) six hours in *M. domestica* it was not possible to determine if there was a circadian rhythm of LPS-induced hyperthermic Tb. However it was of interest to see if the short fever response did have any effect on the normal circadian rhythm of Tb. The Fourier analysis (results for each animal shown in Appendix 11) indicated that the rhythm was disrupted in *M. domestica* when analysing a two day period which involved the LPS-induced hyperthermia.

Table 7.2.4 shows the effect of LPS on the amplitude, acrophase (timing of peak), and period of Tb in each animal. In each animal except MOAG13 a period of 24 hours was determined however the rhythm was not as defined as during control conditions. In addition, other strong periods were also apparent in individual animals as shown in Appendix 11. For example, although the strongest periodogram value was shown at a period of 48 hours in MOAG13, this animal also had strong periods at 24 and 16 hours.

Table 7.2.4 The effects of LPS on Core Body Temperature: Amplitude, acrophase (timing of peak), and period
[period was determined by Fourier analysis; amplitude is the difference between the maximum and minimum Tb during fever; acrophase is given in 24-hour time]

Animal	Condition	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
MOAG13	Saline	33.1	34.3	1.2	16:05	
	LPS	33.7	35.1	1.4	16:06	48
MOAG15	Saline	33.6	34.3	0.7	9:42	
	LPS	33.5	34.8	1.3	9:55; 9:59	24
MOAG16	Saline	33.2	34.8	1.6	9:45; 13:09	
	LPS	33.5	34.8	1.3	14:10	24
MOAG17	Saline	33.7	34.3	0.6	10:08	
	LPS	33.8	34.8	1.0	12:21	*
MOAG18	Saline	34.0	35.2	1.2	9:53	
	LPS	33.1	35.4	2.2	9:49; 18:35	24
PETAG1	Saline	33.7	34.8	1.0	16:27	
	LPS	34.1	35.7	1.6	18:05	24

*insufficient data from MOAG17 to determine period

7.2c Effects of Repeated injections of Bacterial Endotoxin on Core Body Temperature

One *M. domestica* (MOAG13) was subjected to repeated saline/endotoxin paired injections over a five week period. An endotoxin-induced increase in Tb was observed in each treatment. Table 7.2.5 illustrates the latent periods, time affected and mean Tbs obtained from each weekly treatment. It is interesting to note that with repeated injections the time affected increased although latent periods were variable.

The mean endotoxin Tb responses were significantly different from the mean saline values (ANOVA: $p<0.05$; $F=10.7$). In addition, significant differences were observed between saline Tb and endotoxin Tb in each of the five treatments (treatment 1:$p<0.01$; $t=-11.8$; treatment 2: $p<0.01$; $t=-19.9$; treatment 3: $p<0.01$; $t=-23.6$; treatment 4: $p<0.01$; $t=5.0$; treatment 5: $p<0.05$; $t=-2.5$). The fourth treatment of saline followed by endotoxin resulted in quite erratic measurements of Tb with a consistently increased Tb not apparent after endotoxin treatment in this case. Similarly the fifth treatment also showed some inconsistencies compared to the first three treatments. These inconsistencies can be seen in Figures 7.2.3 (a-d).

Figure 7.2.3a Continuous Body Temperature (Tb) Recordings from a *M. domestica* (MOAG13) during a four day period - the effects of a second injection of saline and *E. coli* LPS

[saline was injected at 33.6 hours;LPS was injected at 57.9 hours]

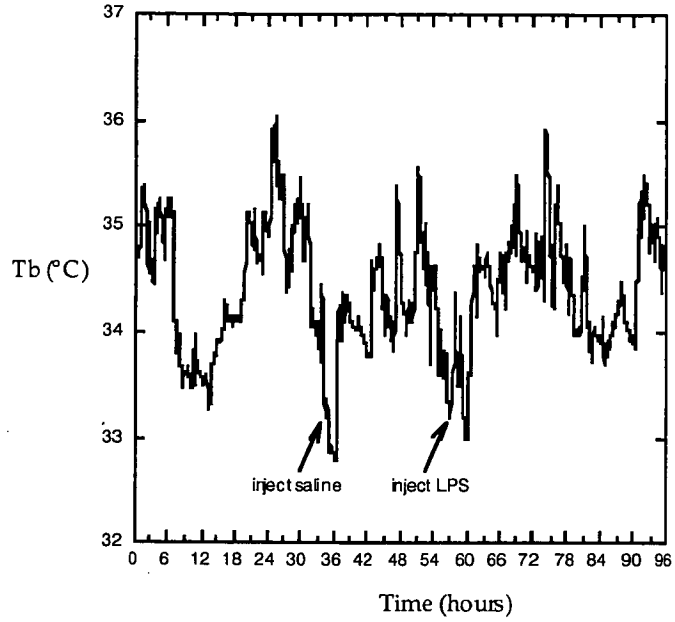


Figure 7.2.3b Continuous Body Temperature (Tb) Recordings from a *M. domestica* (MOAG13) during a four day period - the effects of a third injection of saline and *E. coli* LPS

[saline was injected at 33.7 hours; LPS was injected at 57.5 hours]

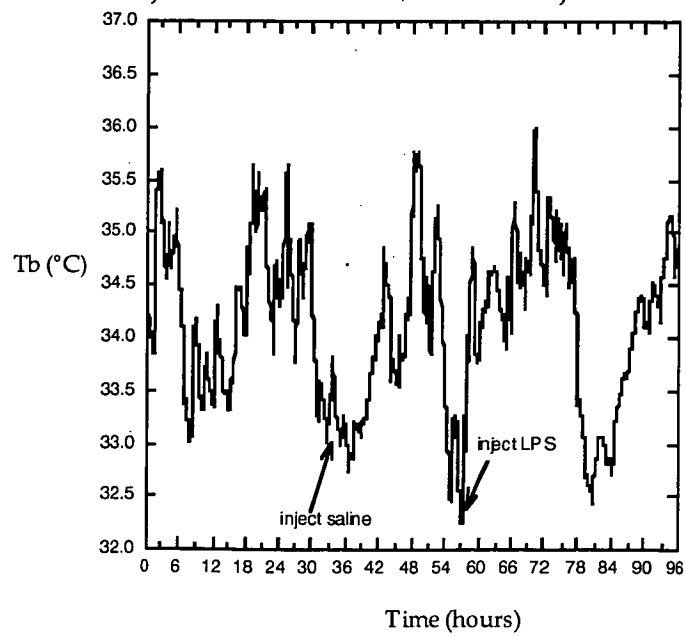


Figure 7.2.3c Continuous Body Temperature (Tb) Recordings from a *M. domestica* (MOAG13) during a four day period - the effects of a fourth injection of saline and *E. coli* LPS

[saline was injected at 33.5 hours; LPS was injected at 58.5 hours]

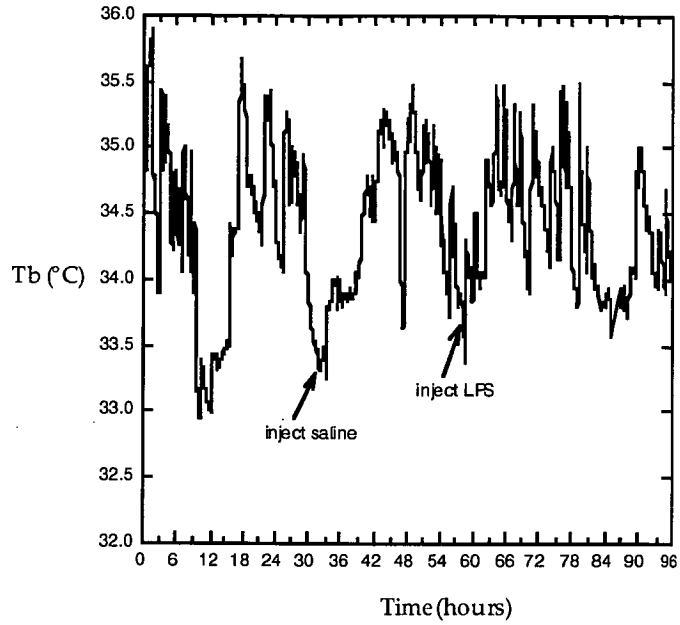


Figure 7.2.3d Continuous Body Temperature (Tb) Recordings from a *M. domestica* (MOAG13) during a four day period - the effects of a fifth injection of saline and *E. coli* LPS

[saline was injected at 33.6 hours; LPS was injected at 57.6 hours]

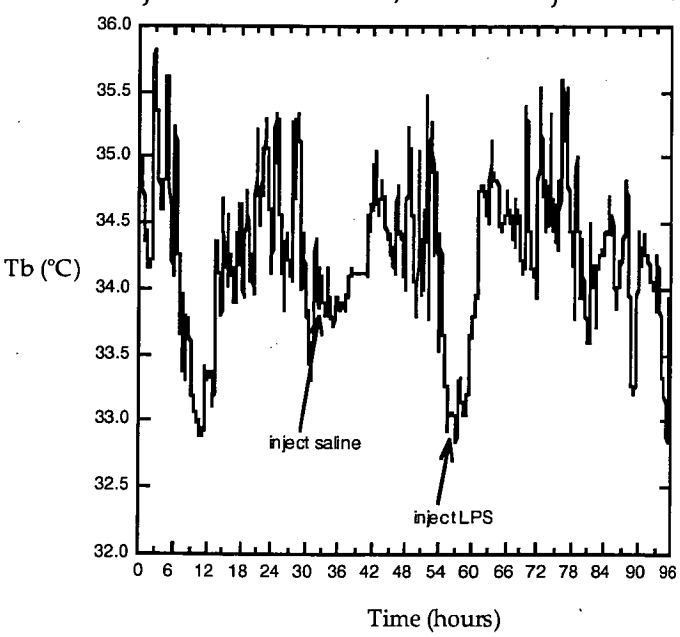


Table 7.2.5 Mean Core Body Temperatures (Tb) of MOAG13 when subjected to repetitive intramuscular injections of Isotonic Saline and Bacterial Endotoxin (*E.coli* LPS) on a weekly basis

Latent period refers to elapsed time before endotoxin affected and time affected refers to the length of time the endotoxin increased the core Tb
ΔTb represents difference between saline Tb and endotoxin Tb
* denotes significance

Condition	Latent Period (mins)	Time affected (hours)	Mean Tb (°C)	ΔTb (°C)
Saline 1	-	-	33.6±0.4	
Endotoxin 1	85	6.5	34.9±0.4	1.2
Saline 2	-	-	34.2±0.4	
Endotoxin 2	135	14.5	34.6±0.4	0.4
Saline 3	-	-	34.0±0.8	
Endotoxin 3	0	19	34.6±0.5	0.6
Saline 4	-	-	34.6±0.4	
Endotoxin 4	210	17	34.7±0.4	0.1
Saline 5	-	-	34.4±0.4	
Endotoxin 5	85	18	34.5±0.5	0.1

The effect of repetitive injections of LPS on normal circadian rhythms of Tb was determined in MOAG13 using Fourier analysis. Although the repetitive fevers were longer in duration than the initial fever produced in MOAG13 and other *M. domestica*, the hyperthermic response to LPS disrupted normal Tb rhythms similarly to that seen in initial LPS responses (see Appendix 11). Table 7.2.6 shows the effect of repetitive LPS injections on the amplitude, acrophase (timing of peak), and period of Tb in MOAG13. As seen during initial fever experiments, the rhythm of Tb was maintained at 24 hours with repetitive LPS injections although the pattern of Tb differed to that seen under control conditions.

Table 7.2.6 The effects of Repetitive LPS Injections on Core Body Temperature in a *M. domestica* (MOAG13): Amplitude, acrophase (timing of peak), and period
[period was determined by Fourier analysis; amplitude is the difference between the maximum and minimum Tb during fever; acrophase is given in 24-hour time]

Condition	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
Saline 1	33.1	34.3	1.2	16:05	
LPS 1	33.7	35.1	1.4	16:06	24
Saline 2	32.8	35.4	2.6	23:19	
LPS 2	33.0	35.5	2.5	21:03	24
Saline 3	32.8	35.8	3.0	00:30	
LPS 3	33.2	36.0	2.8	22:15	24
Saline 4	33.6	35.5	1.9	1:07	
LPS 4	33.4	35.5	2.1	16:07; 17:23	24
Saline 5	33.7	35.3	1.6	03:40	
LPS 5	33.1	35.5	2.4	00:14	24

7.2d Effects of Bacterial Endotoxin on Selected Ambient Temperature

Continuous measurements of selected Tas while undergoing saline and endotoxin treatments are presented in Appendix 6 with a typical response illustrated in Figures 7.2.4a and 7.2.4b. The range Tas selected by *M. domestica* ranged from 15.5 to 38.5°C while 'febrile' compared to 15.2 to 39.1°C under control conditions. Most animals experienced an increase in the mean selected Ta during the 'febrile' phase of increased Tb although one animal selected a lower environmental temperature. Mean selected Tas are presented in Table 7.2.7 for *M. domestica*. PETAG1 selected Tas ranging from 20.2 to 34.7°C while "febrile" compared to 17.46 to 34.0°C under saline control conditions. Continuous measurements of selected Ta while injected with saline and LPS are illustrated in Figures 7.2.4c and 7.2.4d for PETAG1.

Figure 7.2.4a Continuous Recordings of Preferred Ambient Temperature (Ta) from a *M. domestica* (MOAG13) - the effects of an injection of isotonic saline

[*saline was injected at 9.5 hours]

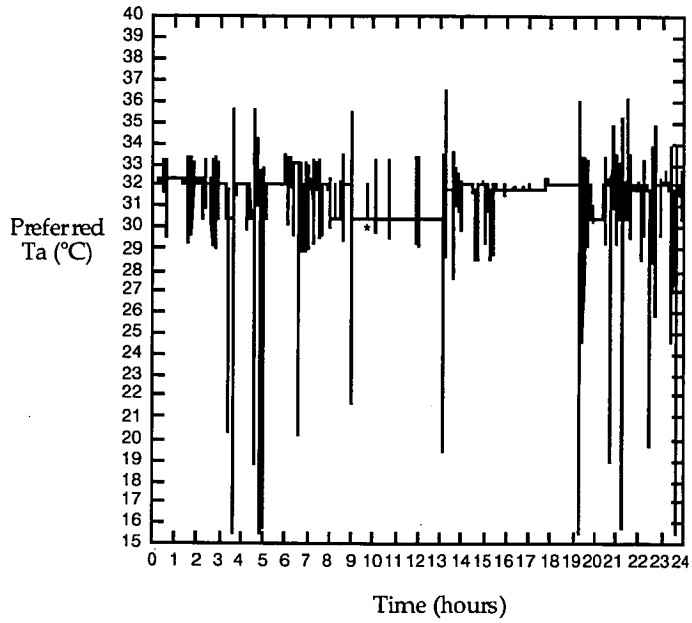


Figure 7.2.4b Continuous Recordings of Preferred Ambient Temperature (Ta) from a *M. domestica* (MOAG13) - the effects of an injection of *E. coli* LPS

[*LPS was injected at 9.5 hours]

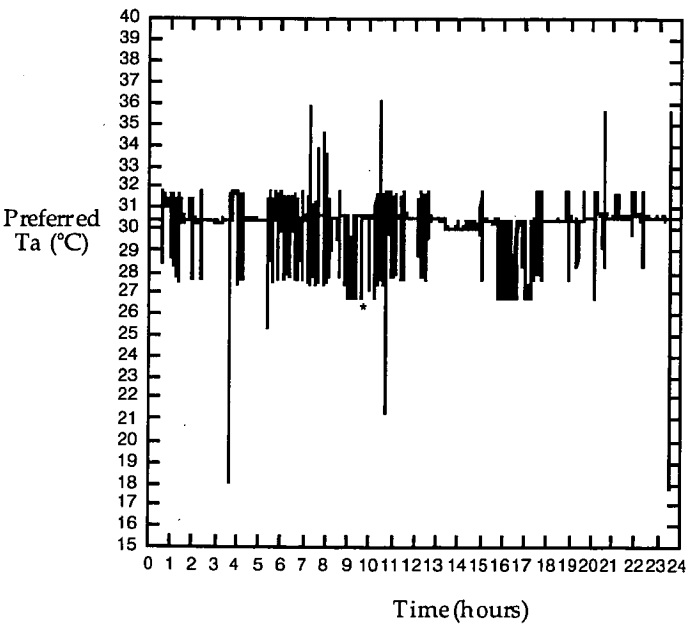


Figure 7.2.4c Continuous Recordings of Preferred Ambient Temperature (Ta) from a *P. breviceps* - the effects of an injection of isotonic saline
[*saline was injected at 13.6 hours]

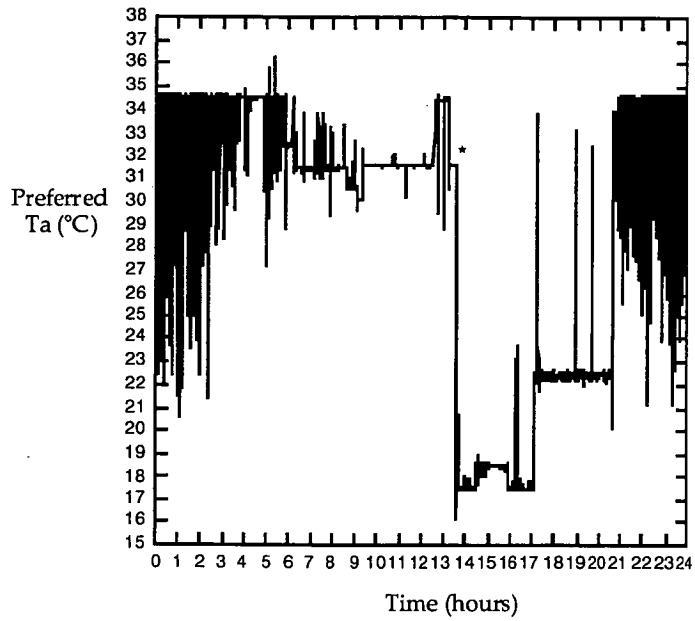


Figure 7.2.4a Continuous Recordings of Preferred Ambient Temperature (Ta) from a *P. breviceps* - the effects of an injection of *E. coli* LPS
[*LPS was injected at 13.2 hours]

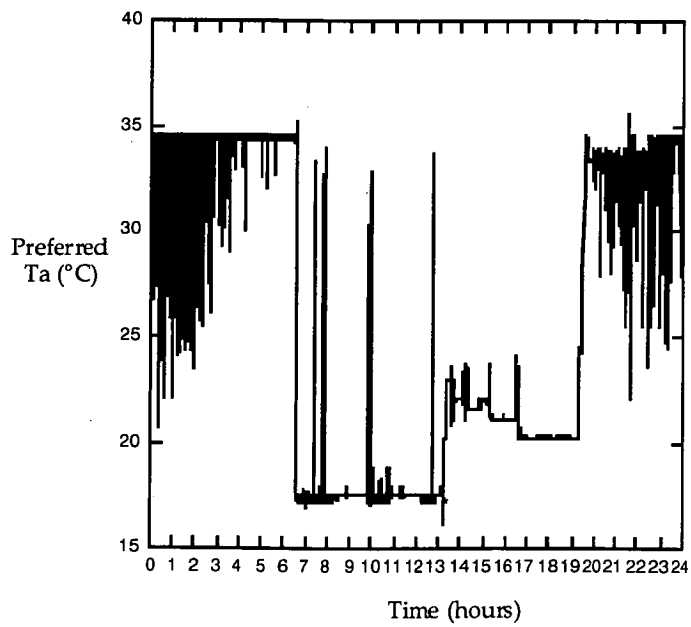


Table 7.2.7 Mean Selected Ambient Temperature (Ta) of *M. domestica* and one *P. breviceps* (PETAG1) in response to intramuscular injections of Isotonic Saline or Bacterial Endotoxin (*E. coli* LPS).

Latent period refers to elapsed time before endotoxin affected and time affected refers to the length of time the endotoxin increased the core Tb
[ΔTa represents difference between selected Ta in saline and LPS conditions]
* denotes significance

Animal	Condition	Selected Ta (°C) (X±SD)	ΔTa (°C)
MOAG13	saline	29.7±1.4	-
MOAG13	*E.coli* LPS	29.8±1.1	0.1
MOAG15	saline	32.1±1.2	-
MOAG15	*E.coli* LPS	32.7±1.4	0.6
MOAG16	saline	31.4±1.6	-
MOAG16	*E.coli* LPS	32.0±1.4	0.6
MOAG17	saline	32.7±1.4	-
MOAG17	*E.coli* LPS	33.2±0.7	0.5
MOAG18	saline	30.0±0.7	-
MOAG18	*E.coli* LPS	28.6±2.0	-1.4
ALL	*saline*	*31.2±1.3*	-
ALL	*E.coli LPS*	*31.3±2.0*	*0.3*
PETAG1	saline	22.6±4.5	-
PETAG1	*E.coli* LPS	23.8±5.5	1.2*

The frequency of responses to each Ta available in the thermal gradient was also determined during control (saline) and LPS conditions for each animal as shown in Figures 7.2.5 (a-f). The frequencies presented have been determined for the time period in which the core Tb was increased due to the LPS. Figure 7.2.6 shows the mean frequency of responses to Ta under control and 'febrile' conditions for *M. domestica* and the *P. breviceps*. There was a tendency for *M. domestica* to select warmer Tas while "febrile" and both species demonstrated a greater variation in Tas selected while 'febrile' compared to control conditions.

Figure 7.2.5a The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in MOAG13

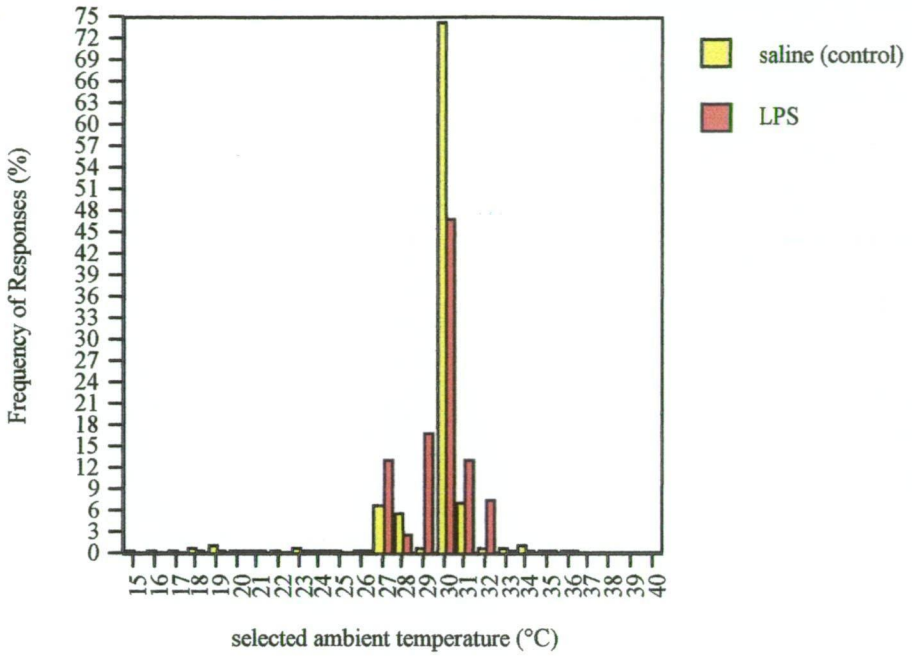


Figure 7.2.5b The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in MOAG15

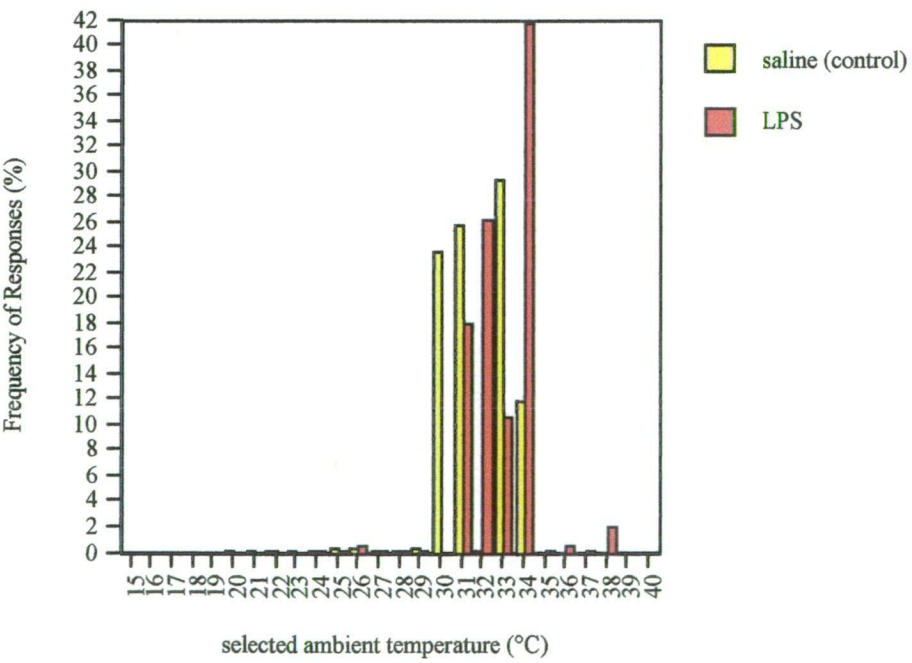


Figure 7.2.5c The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in MOAG16

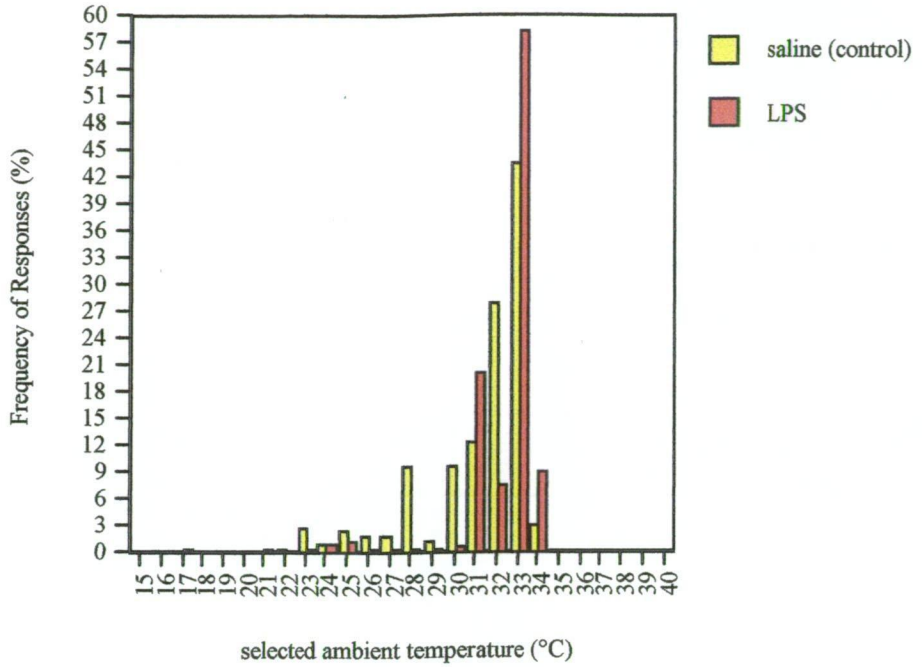


Figure 7.2.5d The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in MOAG17

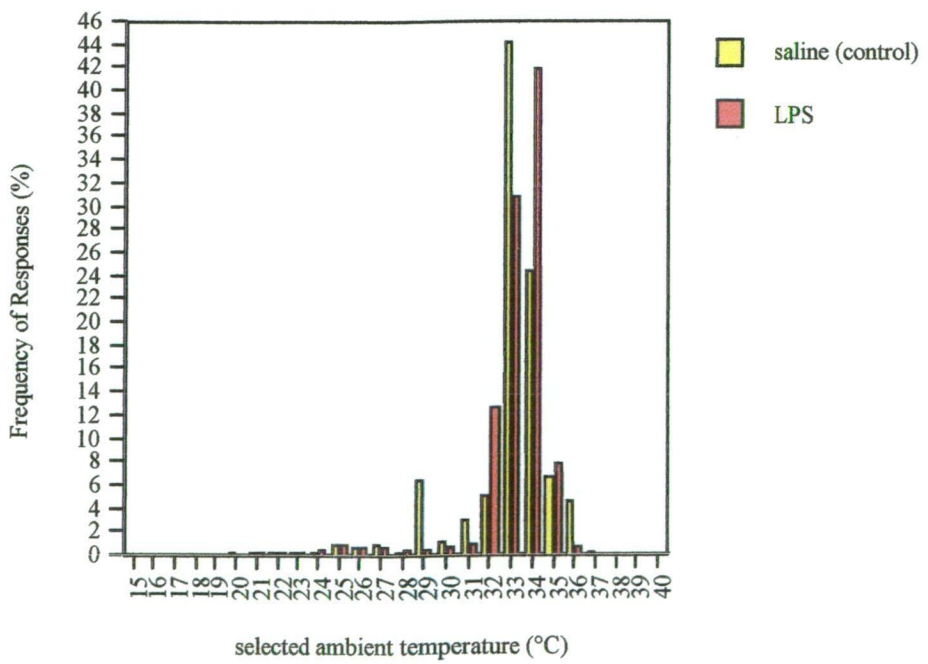


Figure 7.2.5e The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in MOAG18

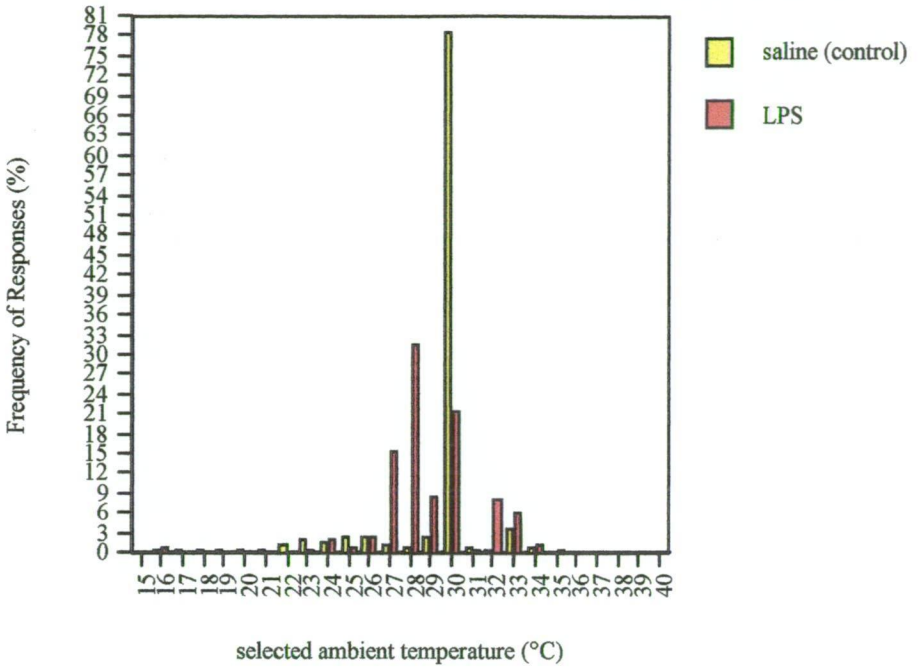


Figure 7.2.5f The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in PETAG1

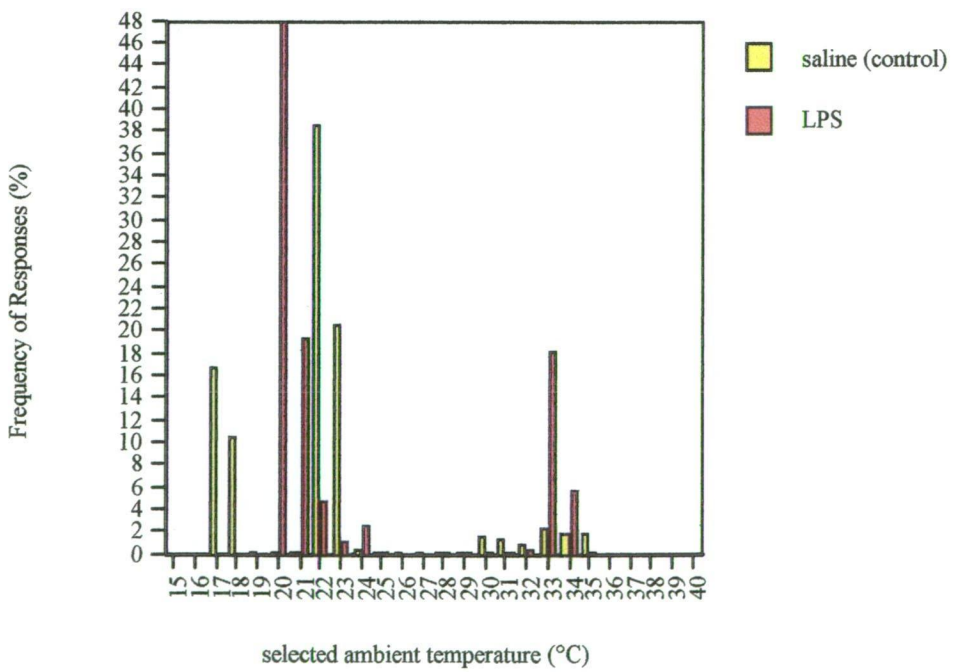
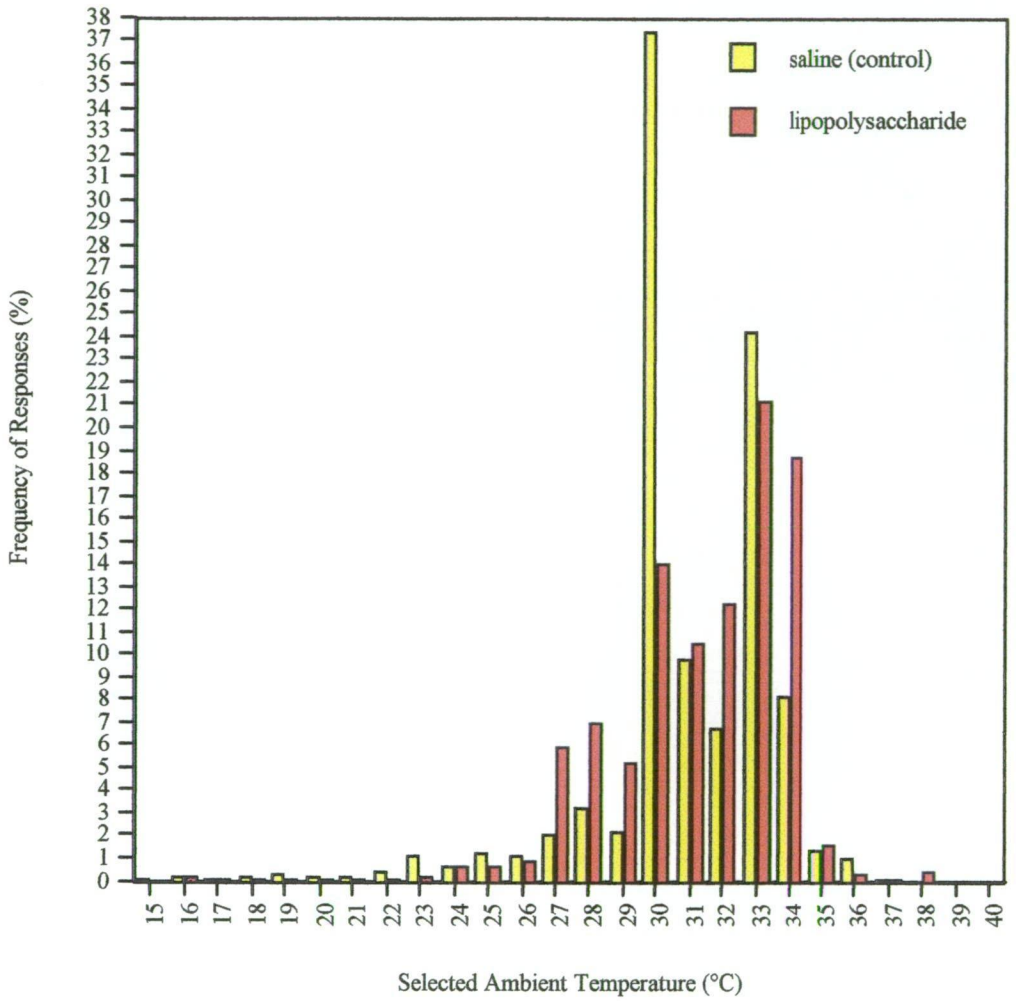


Figure 7.2.6 The effect of lipopolysaccharide (LPS) on the frequency of responses to ambient temperature in *M. domestica* (n=5)



Comparing the mean selected Tas during the time period in which core Tb is elevated, significant differences are noted between individuals (ANOVA: $p<0.05$; $F=14.0$) however no significant difference exists between mean saline Ta and mean endotoxin Ta observed. When considering individual measurements from *M. domestica*, selected Ta was shown to be significantly different after injection of LPS in three out of five animals during the time period that core Tb was elevated (t-test: MOAG16; $p<0.001$; $t=6.5$; MOAG17; $p<0.05$; $t=-0.2$; MOAG18; $p<0.001$; $t=1.4$). Similarly, selected Ta was significantly higher in PETAG1 when core Tb was elevated by endotoxin (t-test: $p<0.001$; $t=22.1$).

As for Tb, the significance of Ta selection was determined from the common time phase in which Tb was elevated by endotoxin in three *M. domestica*. Overall, selected Ta was not significantly affected by endotoxin during this time period (ANOVA: $p>0.05$; $F=1.0$).

Circadian patterns of selected Ta while "febrile" were difficult to ascertain due to the limited time effect of the endotoxin on Tb (i.e, less than 24 hours). Saline and endotoxin treatment days were analysed using Fourier analysis to determine if there was any effect on the overall circadian phases by the endotoxin. Results of this analysis are shown in Appendix 11 for each animal. No distinct circadian effects of endotoxin on Ta selection were apparent in *M. domestica* however there was evidence of a 20 hour rhythm in the *P. breviceps*.

7.2e Effects of Repeated injections of Bacterial Endotoxin on Selected Ambient Temperature

Repetitive endotoxin injections had varying effects on Ta selection in MOAG13 as shown in Figures 7.2.7 (a-h). Table 7.2.8 shows the mean selected Ta for each weekly treatment during the time periods in which Tb is increased due to endotoxin effects. There were no significant differences between these mean selected Tas. Analysis of continuous measurements of selected Ta indicate that selected Ta was not significantly affected by

endotoxin in the first and fifth treatment however significant differences in selected Ta did occur in treatment 2 (t-test; $p<0.001$: $t=66.5$), treatment 3 (t-test; $p<0.001$: $t=-64.1$) and treatment 4 (t-test; $p<0.001$: $t=-28.1$). The frequency of responses to Ta for each repeated saline/endotoxin treatment are illustrated in Figures 7.2.8 (a-d).

Table 7.2.8 Mean Selected Ambient Temperatures (Ta) of MOAG13 when subjected to repetitive intramuscular injections of Isotonic Saline and Bacterial Endotoxin (*E. coli* LPS) on a weekly basis
ΔTa represents difference between saline Ta and endotoxin Ta
* denotes significance

Condition	Mean Ta (°C)	ΔTa (°C)
Saline 1	29.7±1.4	
Endotoxin 1	29.8±1.1	0.1
Saline 2	31.7±1.1	
Endotoxin 2	30.5±1.4	1.2*
Saline 3	29.0±1.7	
Endotoxin 3	30.4±1.5	1.4*
Saline 4	29.9±1.9	
Endotoxin 4	30.9±2.0	1.0*
Saline 5	30.8±1.9	
Endotoxin 5	30.8±1.4	0.0

Figure 7.2.7a Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with isotonic saline for a second time (saline 2)

[*saline was injected at 9.6 hours]

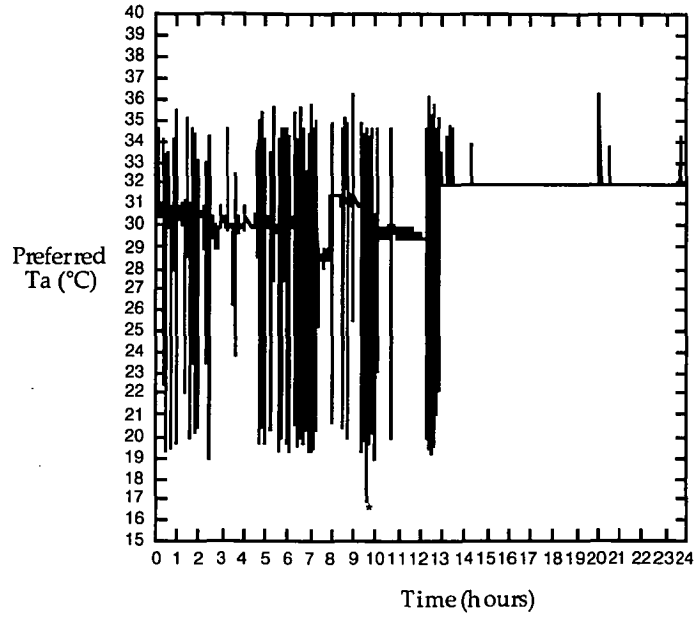


Figure 7.2.7b Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with *E. coli* LPS for a second time (fever 2)

[*LPS was injected at 9.9 hours]

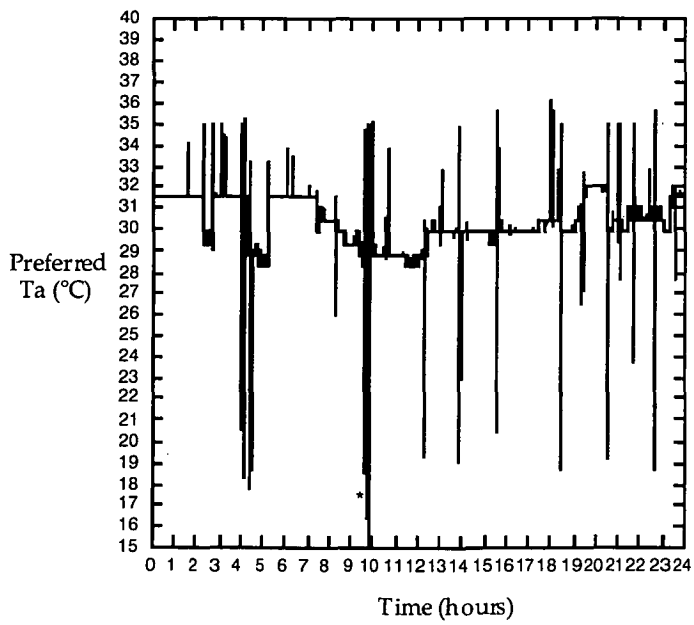


Figure 7.2.7c Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with isotonic saline for a third time (saline 3)

[*saline was injected at 9.7 hours]

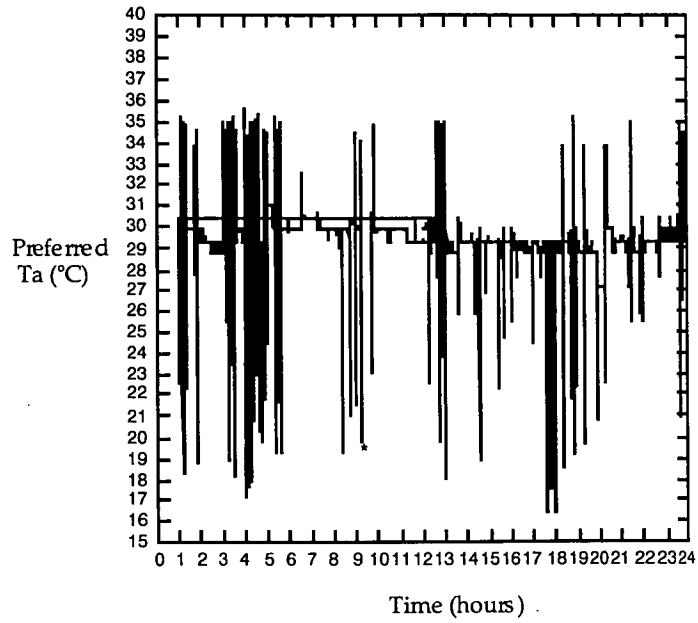


Figure 7.2.7d Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with *E. coli* LPS for a third time (fever 3)

[*LPS was injected at 9.5 hours]

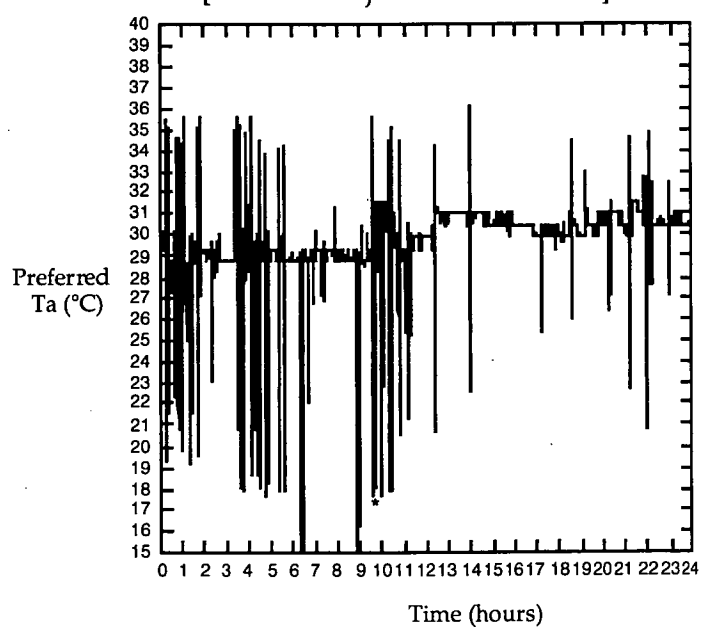


Figure 7.2.7e Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with isotonic saline for a fourth time (saline 4)

[*saline was injected at 9.5 hours]

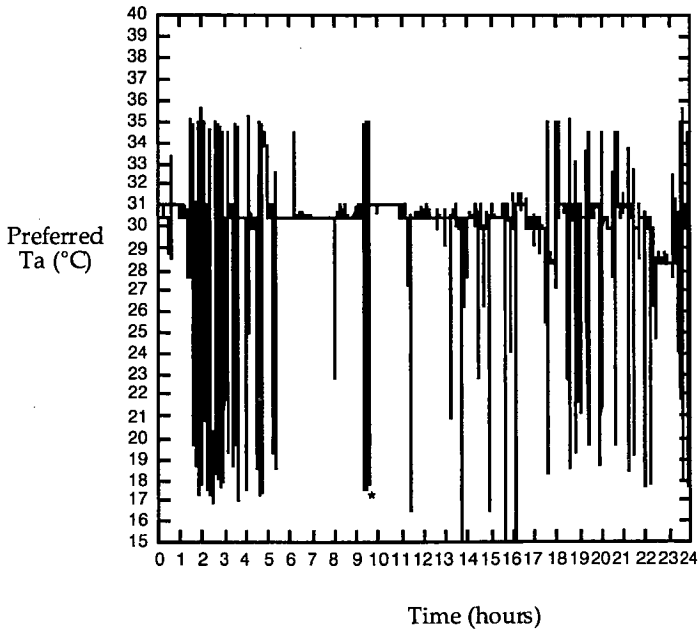


Figure 7.2.7f Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with *E. coli* LPS for a fourth time (fever 4)

[*LPS was injected at 10.5 hours]

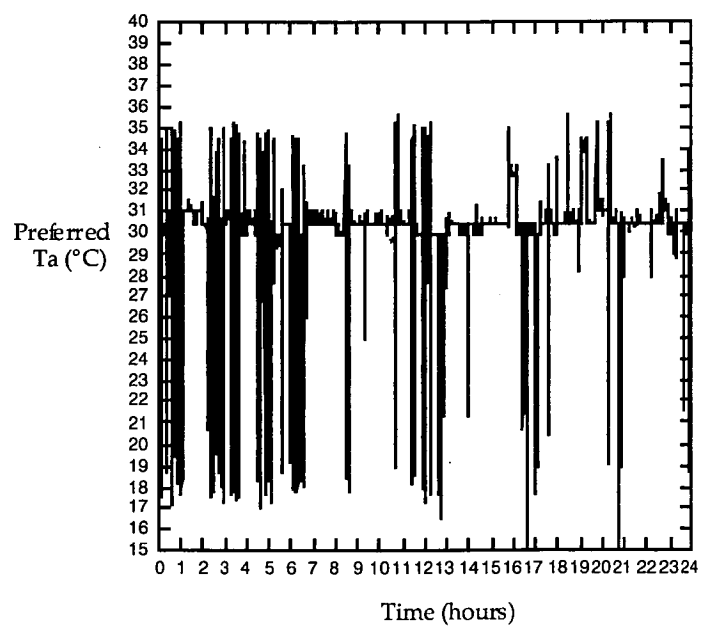


Figure 7.2.7g Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with isotonic saline for a fifth time (saline 5)

[*saline was injected at 9.6 hours]

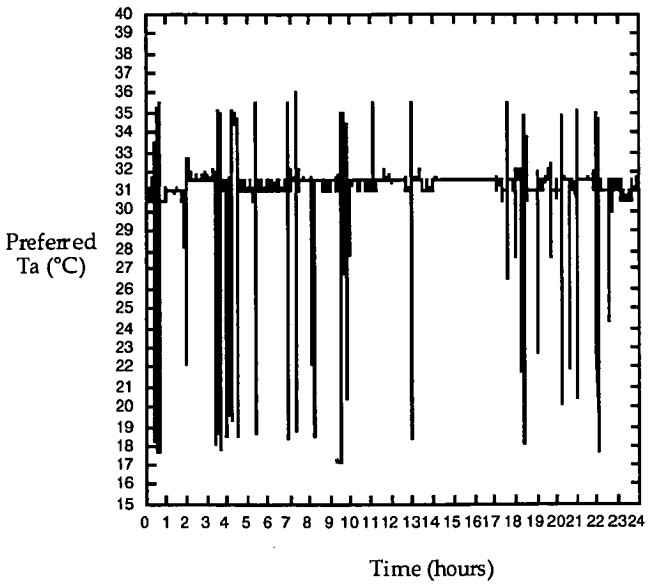


Figure 7.2.7h Continuous Recordings of Preferred Ambient Temperature (Ta) from in MOAG13 when injected with isotonic saline for a fifth time (fever 5)

[*LPS was injected at 9.6 hours]

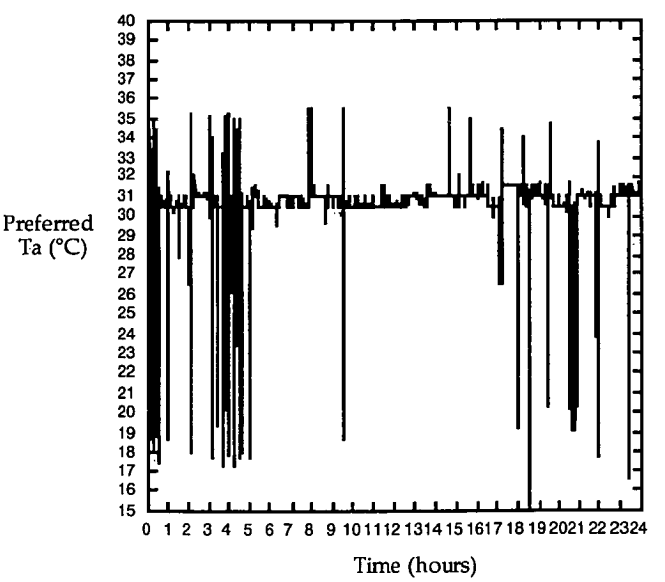


Figure 7.2.8a Frequency of responses to ambient temperature during a second exposure to lipopolysaccharide (LPS) in MOAG13

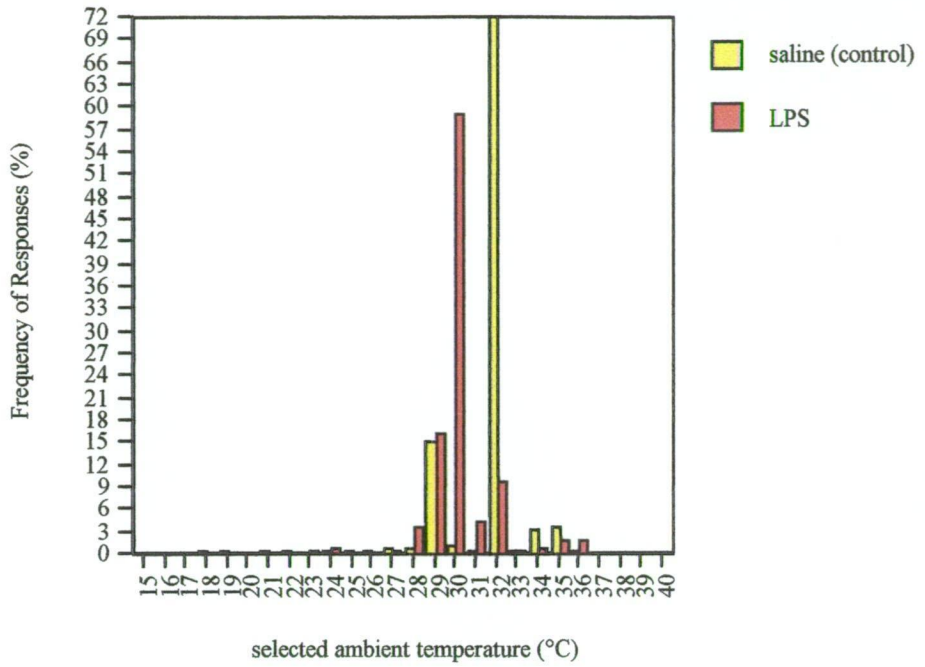


Figure 7.2.8b Frequency of responses to ambient temperature during a third exposure to lipopolysaccharide (LPS) in MOAG13

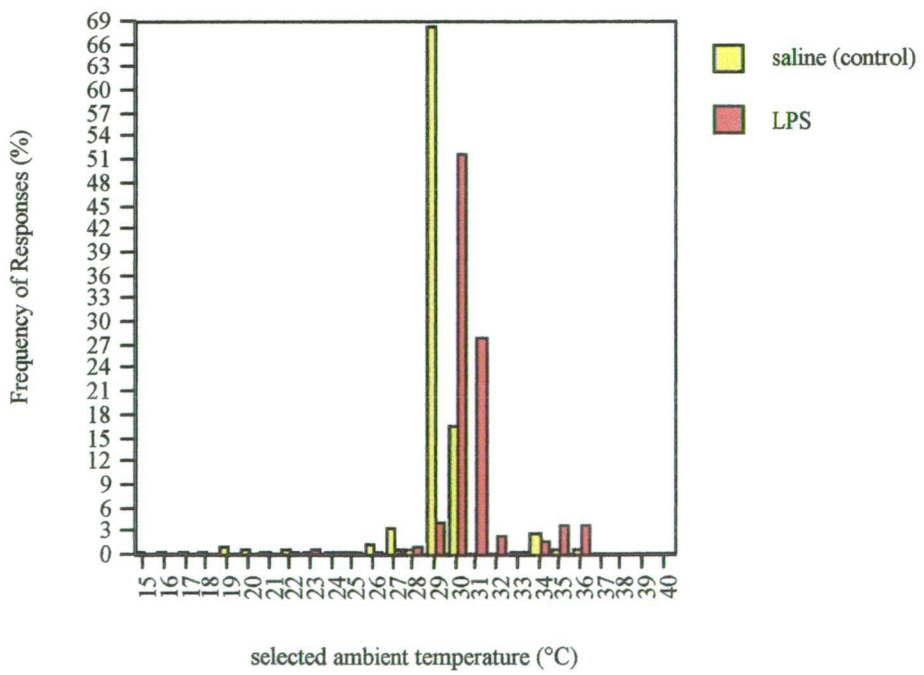


Figure 7.2.8c Frequency of responses to ambient temperature during a fourth exposure to lipopolysaccharide (LPS) in MOAG13

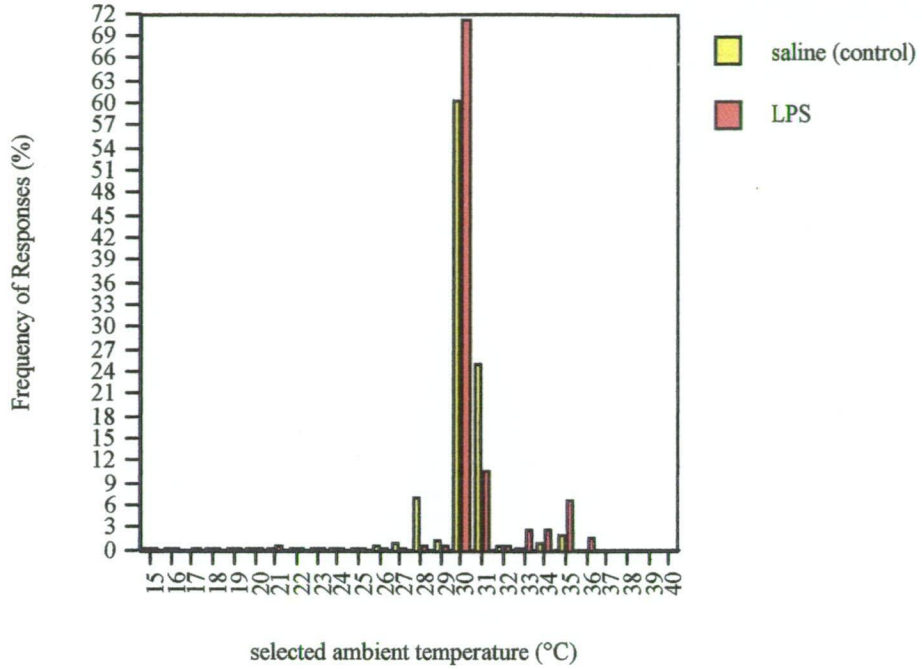
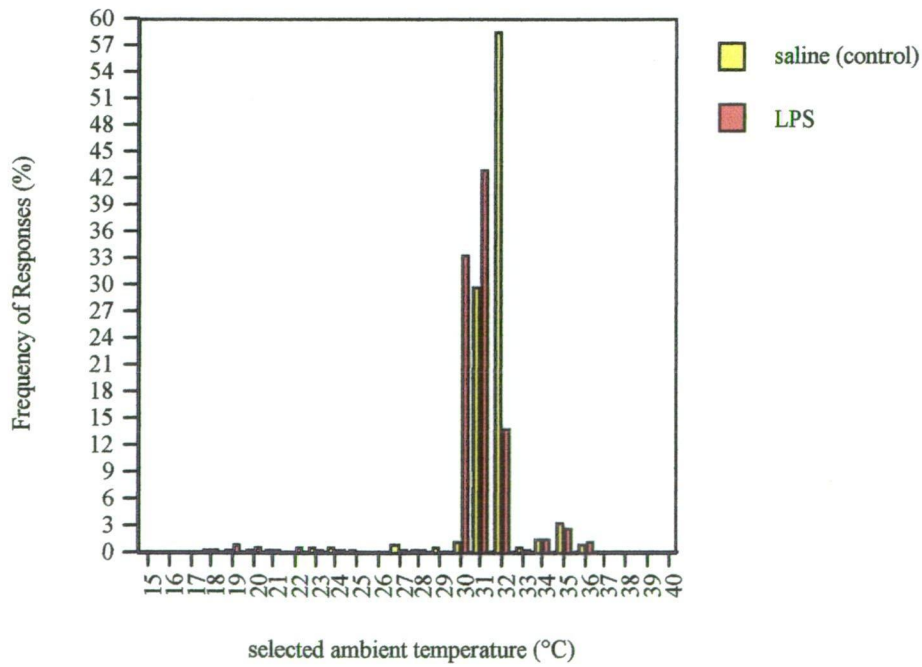


Figure 7.2.8d Frequency of responses to ambient temperature during a fifth exposure to lipopolysaccharide (LPS) in MOAG13



7.3 Discussion

7.3.1 Body Temperature Responses to LPS

Fever is interpreted experimentally as a rise in Tb. The marsupial species, *M. domestica*, like many eutherian mammals, increase core Tb in response to bacterial LPS thus exhibiting a typical febrile response. This increase is of comparable magnitude to that observed in other mammalian species in response to LPS (eg Toien and Mercer, 1998) and is also observed in a *P. breviceps*. However, the increase in Tb observed in *M. domestica* is not sustained for extensive time periods as reported in the rat (Severinsen and Oritsland, 1991) and observed in the brush-tailed possum, *Trichosurus vulpecula* (Nicol, unpublished observations). Instead, a short-term hyperthermic response is observed in *M. domestica* as reported in eutherian mammals such as the rabbit (Moromoto et al., 1988). The magnitude of mammalian fevers often differs between different species (eg Stitt et al., 1985) and so the results indicate that marsupials exhibit typical mammalian febrile responses to LPS.

Febrile responses have been observed in various birds (D'Alecy and Kluger, 1975; Maloney and Gray, 1998) and ectothermic animals (eg Bronstein and Connor, 1984) and so from an evolutionary point of view the present observations are not unexpected. An increased Tb in the host confers an advantage with respect to impaired bacterial growth and improved immune function. Such an advantageous response appears to be the basis of the common origin of fever in invertebrates and therefore vertebrates.

It is often proposed that the hyperthermic response seen in animals following the injection of a pyrogenic substance may be stress related. Cabanac and Laberge (1998) demonstrated that such a response is not evident in goldfish although emotional fever has been observed in lizards (Cabanac and Gosselin, 1993). Similarly, handling has been observed to

increase core Tb in rats (Briese and de Quijada, 1970) and mice (Cabanac and Briese, 1991) indicating that higher animals do exhibit hyperthermic responses when stressed. Saline injections used as a control in this study did not significantly increase Tb in *M. domestica* indicating that such stress from handling is not evident in this species. The hyperthermic response to LPS can therefore be interpreted as evidence of a fever response in this marsupial species.

A varied latent period was observed in *M. domestica* (ie 85-273 mins) which can be interpreted as lengthy in comparison to observations in mammals and birds (eg Maloney and Gray, 1998). However, Pekin ducks exhibit a short latency of fever onset in response to LPS and require a dose of $100\mu gkg^{-1}$ to elicit a fever 24 hours in duration (Maloney and Gray, 1998). Lengthy induction periods for fever have been observed previously in birds and mammals. Pittman et al., (1976) found a lengthy latency of 45-90 mins in response to 0.05-10.0μg of pyrogen injected into the hypothalamus of the chicken. Moreso, LPS administered at doses of 100μg and 1000μg to rabbits increased Tb at 4 hours (240 mins) post-injection with the febrile response lasting 4 hours (Johnson et al., 1996). Long latent periods have been suggested to be due to the formation of an intermediate pyrogen such as a prostaglandin (Pittman et al., 1976) which has been shown to be involved in febrile responses of many species (eg Cooper, 1987). As both *M. domestica* and the *P. breviceps* exhibited long latent periods after LPS injection it is probable that PG may be an integral part of the marsupial fever response.

It is believed that several mediators underly the pathogenesis of fever (Morimoto et al., 1988), although prostaglandins have been proposed as key mediators of pyrogen action within the hypothalamus (Milton and Wendhandt, 1970; Feldberg and Gupta, 1973; Feldberg and Saxena, 1975; Kluger, 1991). The physiological basis of fever is yet to be fully determined but is hypothesised that exogenous pyrogens such as LPS stimulate the production of endogenous pyrogens (such as interleukins) from the host's

immune cells with prostaglandins acting on specific brain sites such as the hypothalamus (Kluger, 1991). However, changes in the rectal Tb of LPS-treated mice in the initial febrile response are not PGE_2-dependant (Abramovich et al., 1994). The widely accepted model of the concept of fever remains that PGE_2 plays a critical role in resetting the hypothalamic set-point for thermoregulation to a higher level. However, additional brain eicosanoids (PGE_2-independent) may also play a critical role in LPS fever with the contribution determined by the specificity of the central mechanisms of fever (Fraifeld and Kaplanski, 1998). The activation of complement is proposed to induce the production of PGE_2 and hence fever (Sehic et al., 1998). Neural control through the vagus nerve is also proposed to be involved in monophasic fevers produced in response to low doses of pyrogen (Romanovsky et al., 1996). Mediators of fever were not analysed in *M. domestica* but it is proposed that the Tb response observed after an injection of LPS in this species support the model of fever involving PG.

The short febrile response observed in *M. domestica* may be attributable to the dosage administered to the animals. Johnson et al., (1996) found that fever was produced for similar durations in rabbits in response to different dosages of endotoxin and that in fact the lower dosage produced a higher Tb. Similarly, small doses of LPS administered to rats cause fever whereas large doses induce hypothermia and shock (Romanovsky et al., 1996). This is also seen in hamsters (Blatteis, 1983), mice (Rips 1989) and ferrets (Romanovsky et al., 1992). Fever-induced changes in Tb has been found to be dose-dependant in many species such as cockroaches (Bronstein and Connor, 1984), ducks (Maloney and Gray, 1998) and mice (Kozak et al., 1994). It is highly likely that sustained febrile responses may be observed in *M. domestica* at higher dosages of LPS, or possibly in response to LPS from an alternative bacterium, or another type of pyrogen. Unfortunately, dose-related experiments could not be performed in this study due to sparse availability of animals and ethical reasons. The use of an alternative pyrogen was not considered necessary as *E.coli* endotoxin has been shown

to be an effective inducer of fever in mammalian species (eg Iriki et al., 1987; Kozak et al., 1994).

Alternatively, the hyperthermic effects of LPS observed in *M. domestica* may be a reflection of the febrile response of aged animals of this species. Four out of five of the *M. domestica* used in the current study were past reproductive age and therefore may exhibit a reduced febrile response typical of aged mammals (eg Tocco-Bradley et al., 1985). Reduced febrile responses have been observed in many mammalian species and have been proposed to be due to alterations in central sensitivity to pyrogen (Clark et al., 1980) or an inability to effectively respond to the cold (Collins et al., 1977; Naylor et al., 1985). Fever is reduced in aged humans and squirrel monkeys (Clark et al., 1980), rabbits (Lipton and Ticknor, 1979; Ferguson et al., 1981; Naylor et al., 1985) and rats (Maitland et al., 1985). Significant differences between young and old rats in response to exogenous *E. coli* are a reflection of the changes in the immune response observed in aged animals according to Maitland et al., (1985). This is supported by the delayed febrile responses observed in aged rats (Fraifeld et al., 1995) and the more dramatic effects of influenza on Tb seen in aged mice (Bender 1996). A recent study by Florez-Duquet et al., (2001) demonstrated LPS fever in both young and aged rats and found that thermoregulatory behaviour (ie. the selection of warmer Tas) while febrile was significant in aged rats but not younger animals. This indicates that LPS may increase the thermoregulatory set-point in aged rats.

Obviously there is an underlying mechanism affecting the ability of aged mammals to respond to fever-inducing agents as a consequence of breakdowns in the aging of the immune system. Prostaglandins are produced at similar levels in young and old mammals (Kaplanski et al., 1994; Fraifeld and Kaplanski, 1998) but the reduced febrile response may be related to decreased sensitivity of thermosensitive neurons to prostaglandins. Strijbos et al., (1993) speculates that impaired febrile responses in aged mice may be mediated by endogenous lipocortin-1 which exerts pyrogenic features. A deficit in the effector mechanisms of the

thermoregulatory system due to aging may be another explanation for the reduced febrile response observed in this study as aged animals are unable to conserve and generate heat (Naylor et al., 1985). However, the deficit in febrile responses in aged rabbits is not attributable to catecholamine-induced thermogenesis (Ferguson et al., 1983) even though catecholamine-induced thermogenesis is reduced in older animals. The specific effects of age on thermoregulation and fever were not a core focus of this study but it is important to remember that aging effects may be apparent particularly in *M. domestica* as most of the animals used were past reproductive age.

Macari et al., (1987) suggest that BAT could be a main site for endotoxin-induced heat production as a significant increase in interscapular BAT temperature in rabbits is observed during pyrogenesis. Similarly, Jepson et al (1988) have shown that endotoxin-induced fever in rats involves sympathetic activation of BAT. Reduced thermogenesis in BAT has been shown by Scarpace et al., (1992) to impair febrile responses in rats. The presence of BAT and the role of NST thermogenesis in marsupials is one of continuing debate. BAT has been shown to be present in a small marsupial species, *S. crassicaudata* with an indication that it may not be necessary for thermoregulation particularly in the short term (Hope et al., 1997). In addition, Rose et al., (1999) have demonstrated noradrenaline-mediated NST in *B. gaimardi*, however this is independent of BAT. The febrile response to bacterial LPS has been shown to involve noradrenaline in rabbits (Bencsics et al., 1995). However, thermogenic responses to noradrenaline have been found to be absent in non-macropod species including *M. domestica* (Nicol et al., 1997) and the marsupial febrile response observed in this study was much smaller (particularly in duration) than mammalian fevers in which BAT has been shown to have a key role in thermoregulation (eg Severinsen and Oritsland, 1991). This indicates that BAT thermogenesis and noradrenaline-mediated NST are not involved in the pathogenesis of fever in non-macropod marsupials such as *M. domestica*.

The fever response exhibited by marsupials appears to be short in duration although there are intraspecies differences. Fever duration in rats is conflicting as lengthy fevers have been observed (Refinetti et al., 1990; Severinsen and Oritsland, 1991) as well as fevers of relatively short duration (eg Sugimoto et al., 1996; Conrad et al., 1997). Generally, prolonged fevers have not been observed in a significant number of studies as most have not utilised radiotelemetric equipment to measure continuous Tb. It is apparent that the use of telemetry to measure Tb in any study of thermoregulation is of paramount importance.

Thyroid hormones have been suggested to be hormone mediators capable of modulating thermoregulatory circuits and altering the thermal set-point in endotherms. In response to LPS, T_4 levels increase in rabbits indicating that an increased input of hypothalamic TRH may contribute to an elevation in the thermoregulatory set-point and thus characteristic hyperthermic response of fever (Riedel, 1983; Riedel, 1990; Keil et al., 1996). Similarly, in rats plasma thyroid hormones modulate febrile responses to endotoxin (Coelho et al., 1992). T_4 and T_3 levels were not measured from *M. domestica* or the *P. breviceps* during fever in this study however it can be assumed that they may play some role in the observed hyperthermic response given the role of thyroid hormones in marsupial thermoregulation.

7.3.2 Sensitivity to LPS

Tolerance to LPS was injected i.m. at 1mg/kg every seven days for five weeks and analysed in one *M. domestica*. Interestingly, the LPS produced a longer febrile response with repetitive treatments rather than reducing the degree of LPS-induced hyperthermia. This observation is distinctly different to previous studies in guinea pigs, rats and rabbits where tolerance to endotoxin is noted. Roth et al., (1994) showed that i.m. injections of 20μg/kg LPS every 3 days results in a gradual disappearance of the febrile response in guinea pigs. The same dose and route of administration in rats results in a sudden disappearance in the febrile response (Rosenthal et al., 1996). An abrupt and complete development of

tolerance to endotoxin has also been observed in rabbits when injected (i.v.) on two consecutive days with 10 μg/kg LPS (Wakabayashi et al., 1994). The higher dose used in this study may explain why tolerance was not observed as higher doses of LPS (ie 50 μg/kg, 100 μg/kg and 1000 μg/kg; all i.m.) have been found to have no effect on the first phase of fever in rats (Wilkinson and Kasting, 1990; Wilkinson and Pittman, 1994; Rosenthal et al., 1996). However all three of these studies observed a reduced fever response in the second phase of fever. A weakened febrile response has also been observed in rabbits in response to repeated injections (i.v.) of a low dose (0.1 μg/kg) of LPS on five consecutive days with the magnitude of the fevers from the second and all subsequent injections quantitatively indistinguishable from one another (Goelst and Laburn, 1991).

Interestingly, behavioural selection of Ta while febrile did alter with repetitive treatments of endotoxin. This thermoregulatory behaviour was particularly prominent in the second, third and fourth treatments and may account for the increase in fever observed with subsequent treatments. By selecting a higher Ta the set-point of thermoregulation may be increased to a higher level and maintained with the concurrent hyperthermic behaviour. The homeostatic set-point had been increased by the final treatment thus behaviour was no longer as prominent but a hyperthermic response to LPS could still be maintained for a lengthy period.

Rosenthal et al., (1996) suggest that there is a markedly reduced sensitivity to LPS after the initial injection with effects of further injections dependant upon the administration route and dosage of LPS. This is supported by the evidence of reduced or completely suppressed cytokine release in response to repeated injections of LPS (Wakabayashi et al., 1994). The metabolic effects of LPS are mediated by cytokines (such as interleukin-1) released endogenously. The attenuation or suppression of these mediators when LPS is repeatedly injected may be the basis of similar effects (ie attenuation or suppression) on fever observed in response to repeated

injections. According to Wakabayashi et al., (1994), changes in febrile responsiveness to successive LPS injections is closely associated with similar changes in circulating levels and cellular production of these cytokines.

A recent study by Kamerman et al., (2001) has shown that heat-stressed rats develop tolerance to LPS despite a normal febrile response to their initial LPS injection. These authors speculated that this tolerance may have been due to the large doses of LPS used (ie. 100μg/kg injected intraperitoneally) or a decrease in cytokine production due to the heat exposure. The *M. domestica* used in this study were from a heat-acclimated colony where the Ta was 28-30°C whereas the rats in the study by Kamerman et al., (2001) were exposed to a regulated Ta of 40°C. Therefore the differences in Ta and dosage of LPS would explain the differences observed in LPS tolerance. Interestingly, a study by Kluger et al., (1997) found that heat stress amplified the febrile response of rats. These authors also exposed the animals to a Ta of 40°C but used LPS dosages that were much lower than those used by Kamerman et al., (2001). Both of these studies do indicate that exposure to higher Tas does affect febrile response to LPS although the underlying mechanisms remain unknown. Presumably the acclimation of *M. domestica* to a warm environment would also have some effect on the febrile response to LPS and may also affect the development of LPS tolerance in this animal.

In the *M. domestica*, the magnitude of the fever to repeated injections of LPS does not weaken although the final two fevers observed increased Tb less than after the previous treatments. This lack of sensitivity to LPS indicates that either cytokine release is not affected or another underlying factor is involved or behaviour is compensating for underlying metabolic and physiological effects of LPS in marsupials. Unfortunately, only one animal was subject to this treatment due to ethical and time constraints and so the evidence is inconclusive. However, it does highlight the possibility that although higher vertebrates probably share a common origin of fever

there may be differences in some of the specific underlying physiological and metabolic factors in different species.

The relatively high dose of LPS used in this study results in no apparent attenuating effects of the febrile response. The development of a more prolonged response to repeated LPS injections may be due to the initial injection sensitising central receptors near the hypothalamus which stimulate prostaglandin production as prostaglandins are believed to be key mediators in the second phase of fever (Kluger, 1991). An initial injection of LPS most likely produces a monophasic fever which stimulates the release of endogenous pyrogens or prostaglandins which act centrally within the brain. Unfortunately, circulating prostaglandin levels were not measured following LPS administration and the role of prostaglandins in marsupial fever is unknown.

7.3.3 Behavioural Thermoregulation when Febrile

The selection of a higher mean environmental temperature while febrile did occur in most of the animals studied. However, this increase was not significant for *M. domestica*. In addition, patterns of temperature selection during hyperthermic LPS-induced Tb demonstrate a tendency for animals to seek a variety of Tas. These observations do not fully support the set-point theory of thermoregulation. While febrile, due to a consistently raised core Tb, *M. domestica* would be expected to thermogenically adjust by increasing the hypothalamic set-point and thus seek warmer environmental temperatures. This was observed to some degree however the lack of significance and consistent evidence suggests that either behaviour is not an integral part of the fever response or the hypothalamic set-point is not elevated during fever in this animal. On the other hand, the observation that the *P. breviceps* did utilise thermoregulatory behaviour while febrile does support the set-point of thermoregulation as this animal tended to select higher temperatures when in LPS-induced hyperthermia. As only one *P. breviceps* was studied results are inconclusive and can only be used as a comparison to those obtained from *M. domestica*.

Many ectotherms (and some endotherms) have previously been shown to produce behavioural fevers in response to pyrogens but this study is the first observation of such a response in marsupials. Behavioural fever has been established in a variety of ectotherms (Cabanac, 1990) although not all ectotherms produce fever in response to pyrogens (Laburn et al., 1981; Marx et al., 1984; Adamo, 1998). In response to bacterial endotoxin, newborn rabbits select a warmer Ta until one hour post-injection when a cooler area is selected corresponding to changes in core body temperature (Szekely, 1984). Blatteis and Smith (1980) found that febrile guinea pigs select warmer environments while Tb is elevated. Similarly, mice in temperature gradients select warmer Tas and exhibit higher Tbs within 90 minutes after LPS administration (Akins et al., 1991). However, gerbils choose lower Tas while febrile (Akins and Thiessen, 1990). Cold-seeking behaviour has also been observed in rats when hypothermic through endotoxin exposure (Romanovsky et al., 1996). It is possible that like the gerbil and to some degree the newborn rabbit, laboratory-bred marsupials limit increases in Tb by behavioural selection of lower environmental temperatures. Mammals in their natural habitat however may seek warmer temperatures while febrile (as seen in the *P. breviceps*) however such field studies are yet to be undertaken.

Period of day is likely to be an influencing factor in behavioural thermoregulation during fever as rats have been shown to gradually increase their selected Ta as fever develops during the dark phases of the day with no change in selected Ta seen while animals are febrile during the light phases (Sugimoto et al., 1996). Similarly, a more intense febrile response has been observed in pigeons during nocturnal hours compared to diurnal hours (Nomoto, 1996). As LPS injections were always administered in the light phases of the day in this study it is likely that this may be directly involved in the lack of evidence for heat-seeking behaviour while febrile. From the experimental evidence it appears that the elevation of the hypothalamic set-point does not occur in *M. domestica* which may reflect the absence of NST in this species or be a reflection of its status as a laboratory-

bred animal maintained in constant conditions. However, fever should be induced in the dark phases of a day to ascertain this view.

Obviously the importance of behavioural thermoregulation in the normal maintenance of body temperature in marsupials will have some decisive effect in how fever affects temperature seeking behaviour. As seen in Chapter 3, environmental temperature appears to have no circadian basis with respect to Tb regulation however selected Ta tends to be out of phase with Tb under normal conditions. The importance of the environment is very much dependent on the type of animals used. Laboratory-bred animals maintained at constant conditions (such as *M. domestica* in this study) do not utilise Ta to thermoregulate like a species bred in the wild (see Chapter 3). The ability of a laboratory-bred animal to utilise Ta to enhance or reduce a typical hyperthermic febrile response is therefore limited.

As noted by Gordon (1993b), most studies on febrile rodents have been investigated at relatively cool room temperatures (often 10°C lower than the selected temperature range of the animal). This may have dramatic consequences on febrile responses observed as at lower Ta an alteration in the thermoregulatory set-point may occur affecting the magnitude of any induced fever response. According to Satinoff and Henderson (1977) the height of fever is lowered in the cold. However, Conrad et al., (1997) observed a monophasic response to bacterial LPS in rats independent of Ta with monophasic responses exhibited at low and normal Ta but not at high Ta (although at high Ta, Tb was increased). Conversely, fever-induced cats exposed to warm temperatures (ie 30°C and 37°C) show a monophasic fever whereas a biphasic fever is usually observed at room temperature (Amini-Sereshki-Kormi, 1996). This reflects the need for the relative Ta of experimental conditions to be considered in any fever studies in both endotherms and ectotherms.

The evidence of a typical monophasic mammalian febrile response in at least two marsupial species contributes to the consensus of a common

phyletic origin of fever in the higher vertebrates. Although the response seen indicates some differences to that observed in some mammals and birds there are variations in duration and peak febrile temperatures among higher vertebrates generally. With repeated injections of LPS, *M. domestica* exhibited a heightened febrile response assisted by thermoregulatory behaviour and tolerance to endotoxin was not noted.

The results from this study indicate that marsupials generally fit into the ranges of fever so far reported in the literature for mammals and birds. The inability of some marsupials to behaviourally use the environment as a significant contributor to their hyperthermic response to LPS indicates that their increase in Tb is probably determined through autonomic regulators with some small contribution from the ambient conditions. However when exposed to LPS on a repetitive basis behaviour may be utilised. The *P. breviceps* studied did utilise behavioural thermoregulation while febrile which supports the set-point theory of thermoregulation. *M. domestica* however, does not demonstrate such a reliance on temperature-seeking behaviour while febrile although the animals of this species were laboratory-bred which may explain this difference. As few investigations have been concerned with temperature-seeking behaviour of endotherms during fever, information about behavioural thermoregulation, particularly during fever, is limited. The conflicting results of this study with some of the available evidence in rodents suggests that more research is warranted in this area.

CHAPTER 8

THERMOREGULATORY RESPONSES TO HYPOXIA

8.1 Introduction

Animals exposed to hypoxia, either through a reduction of oxygen carrying capacity or by lowering ambient oxygen concentrations, generally exhibit a variety of physiological responses including increased ventilation and cardiac output, reduced core Tb and altered thermoregulatory behaviour (Wood, 1991). This typical hypothermic response is beneficial physiologically (as it reduces oxygen demand at rest) however whether this response involves a functional shift in the set-point of thermoregulation remains unclear.

The magnitude of hypoxic hypometabolism is dependant upon a number of parameters including Ta, gender and/or size of the animal and the degree of hypoxia (Gautier, 1996). Small mammals (both newborn and adult) exhibit a depression of metabolic rate in response to acute hypoxia (eg Saiki and Mortola, 1996) however such a response is not always observed in larger mammals (eg Korducki et al., 1994). Typically, during hypoxia, a reduced Tb is observed in mammals at Ta values below thermoneutrality (eg Dupré et al., 1988) whereas at Tas above thermoneutrality no change in Tb is observed (Hill, 1959). This highlights the importance of ambient temperature with respect to endothermic thermoregulation particularly during limited oxygen availability. The magnitude of a hypoxic hypometabolism response has also been shown to be inversely related to the resting oxygen consumption of a species (Frappell et al., 1992) which explains the differences observed between hypoxic responses in large and small animals.

The decrease in Tb observed during hypoxia is proposed to be regulated as a number of hypoxic animals have been shown to use behaviour to thermoregulate at a lower Tb. A variety of ectotherms and endotherms including the crayfish and salamander (Dupré and Wood, 1988), lizard (Hicks and Wood, 1985; Wood et al., 1987), and mouse, rat and hamster (Gordon and Fogelson, 1991) select cooler Tas while hypoxic indicating a decrease in the thermal set-point as the behavioural hypothermia appears to be regulated (Wood, 1991). This reduction in the set-point of thermoregulation during hypoxia is mediated through autonomic and/or behavioural parameters including an inhibition of the central nervous system which results in a loss of shivering and non-shivering thermogenesis (eg Gautier et al., 1991). However, the rat has also been shown to select warmer Tas despite a cooler Tb while hypoxic (Dupré and Owen, 1992). Further studies to identify the importance of behavioural thermoregulation during hypoxia in mammals is therefore warranted both in eutherian and non-eutherian species. According to Gautier (1996) the hypothalamus is the key control centre of thermoregulatory responses to hypoxia and there is significant evidence that the thermal set-point is lowered during hypoxia through direct input to neural structures.

Changes in core Tb (via temperature-sensitive transmitters) and selected Ta (via a thermal gradient) in response to hypoxia in *M. domestica* and was investigated in this study. It was hypothesised that both of these species would exhibit a drop in core Tb and select cooler Tas while exposed to low ambient oxygen concentrations. Basal metabolic rates were also investigated in *M. domestica* and the effects of Ta on BMR assessed at various Tas under normoxic and hypoxic conditions. It was hypothesised that *M. domestica* would have a BMR similar to that previously reported for other marsupials and that hypoxia would reduce BMR in this species. Tb and Selected Ta in a *P. breviceps* under hypoxic conditions were also analysed for comparison.

8.2 Results

8.2.1 Effects of hypoxia on circadian patterns of core body temperature and selected ambient temperature

Mean core Tb over 24 hours was significantly reduced by hypoxic conditions ($F=32.7$; $p<0.01$) in *M. domestica* as illustrated in Table 8.2.1. Simultaneous selections of Ta were not dramatically affected by hypoxia in *M. domestica* (see Table 8.2.2). Mean selected Ta over a 24 hour period for *M. domestica* was 29.3±1.4°C during hypoxia compared to 29.9±0.9°C under control (normoxic) conditions of 21%O_2.

Table 8.2.1. Effects of Hypoxia on twenty-four hour means of Core Body Temperature (Tb) in *Monodelphis domestica*
Tb was measured under normoxia (21% O_2) and hypoxia (12-15% O_2) and mean±SD was calculated from 24 hours of recording

Animal	Normoxia Core Tb (°C)	Variance	Hypoxia Core Tb (°C)	Variance
MOAG13	34.5±0.5	0.2	33.8±0.5	0.2
MOAG15	34.3±0.6	0.8	33.5±0.7	0.4
MOAG16	34.6±0.4	0.3	34.1±0.3	0.2
MOAG17	34.3±0.6	0.5	33.9±0.6	0.3
MOAG18	34.8±0.6	0.3	33.7±0.4	0.2
Average	*34.5±0.2*	*0.4*	*33.8±0.2*	*0.3*

Core Tb was reduced in the single *P. breviceps* during hypoxia (from 34.8±0.5°C to 33.9±0.5°C). The *P. breviceps* selected a mean 24-hour Ta of 26.5±7.1°C while hypoxic compared to a normal mean selected Ta of 33.58±1.7°C. The selection of a lower Ta during hypoxia was insignificant in *M. domestica* ($F=1.5$; $p>0.5$) however it was shown that there was more variance in selected Ta under control conditions than during hypoxia over a 24-hour period (Table 8.2.2). The mean Selected Ta was also significantly lower during hypoxia in the *P. breviceps* ($t=103$; $p<0.005$).

Table 8.2.2. Effects of Hypoxia on twenty-four hour means of Selected Ambient Temperature in *Monodelphis domestica*

Selected Ta was measured from each animal while in a longitudinal thermal gradient under normoxia (21% O_2) and hypoxia (12-15% O_2) and mean±SD was calculated from 24 hours of recording

Animal	Control Selected Ta (°C)	Variance	Hypoxia Selected Ta (°C)	Variance
MOAG13	30.8±1.3	1.8	29.9±1.8	3.2
MOAG15	29.3±2.2	4.7	29.3±1.7	2.9
MOAG16	28.4±1.4	1.9	28.1±1.4	2.0
MOAG17	31.1±3.6	12.7	31.6±2.0	3.8
MOAG18	28.6±2.9	8.2	27.5±2.5	6.6
Average	*29.7±1.3*	*5.8*	*29.3±1.4*	*3.7*

Appendix 7 shows continuous recordings of Tb during normoxia and hypoxia for each animal. Figures 8.2.1a and 8.2.1b illustrates a typical Tb recording of one *M. domestica* and PETAG1 during normal and hypoxic conditions. Tb recordings were analysed using Fourier analysis and the effects of reduced oxygen levels on circadian rhythms of Tb determined. Data were analysed using a single series Fourier analysis and results plotted by period using transformations in which the mean was subtracted and detrended and period values determined. Analyses of individual animals are given in Appendix 12. Table 8.2.3 shows the effects of hypoxia on the amplitude, acrophase (timing of peak), and period of Tb in each *M. domestica*. The normal circadian rhythm was not apparent during exposure to hypoxia in this species with circadian rhythms of Tb severely disrupted in

Figure 8.2.1a Continuous Recordings of Body Temperature (Tb) from a *M. domestica* (MOAG18) over a three day period - the effect of hypoxia
[exposure to hypoxia was between 12.6 hours and 36.6 hours]

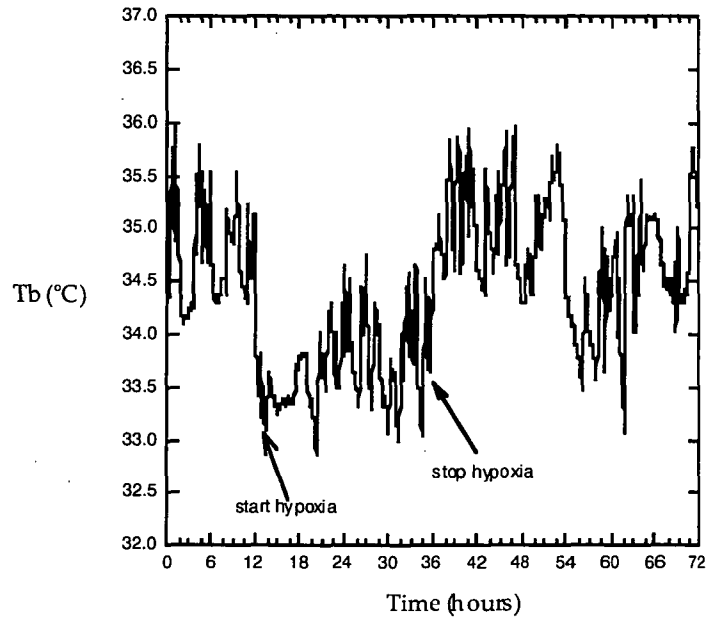
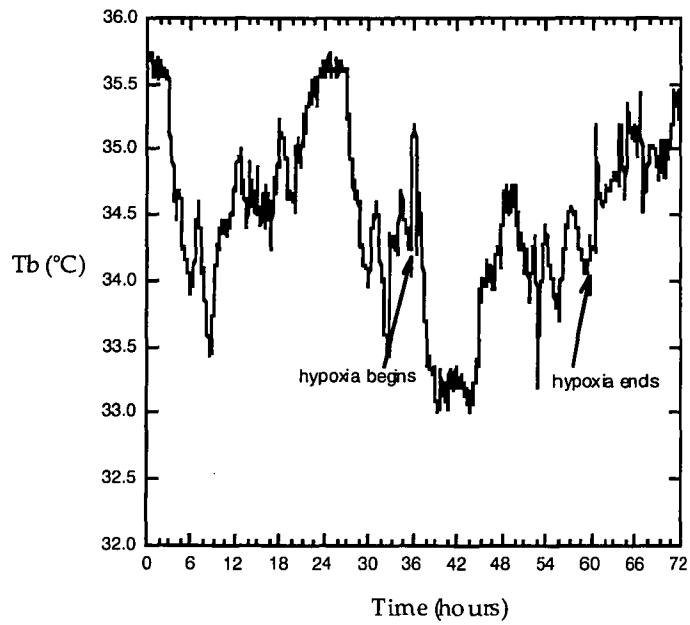


Figure 8.2.1b Continuous Recordings of Body Temperature (Tb) from a *P. breviceps* over a three day period - the effect of hypoxia
[exposure to hypoxia was between 36.2 hours and 60.2 hours]



M. domestica with periods ranging from 12 to 48 hours. A rhythm of 12 hours was noted in the *P. breviceps* during hypoxia. Animals showing 24 hour periods did not show a distinct rhythm as seen under control conditions (see Appendix 12). In addition, other rhythms were also apparent in individual animals. For example, MOAG17 also had strong rhythms at 48 hours and 8 hours as shown in Appendix 12.

Table 8.2.3 The effects of Hypoxia on Core Body Temperature: Amplitude, acrophase (timing of peak), and period
[Period was determined by Fourier analysis; amplitude is the difference between the maximum and minimum Tb during hypoxia; acrophase is given in 24-hour time]

Animal	Condition	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
MOAG13	Normoxia	31.6	35.7	4.1	00:33; 00:45; 06:07	24
	Hypoxia	32.7	34.8	2.1	15:54	24
MOAG15	Normoxia	33.0	35.5	2.5	04:04; 04:47	24
	Hypoxia	32.1	34.7	2.6	23:54; 23:58; 00:00	24
MOAG16	Normoxia	33.3	35.9	2.6	2:59	24
	Hypoxia	33.5	34.7	1.2	15:19	12
MOAG17	Normoxia	32.7	35.7	3.0	00:20	24
	Hypoxia	32.3	34.5	2.2	23:06	24
MOAG18	Normoxia	32.5	36.4	3.9	04:41	24
	Hypoxia	32.9	35.0	2.1	11:42	48

Initial reactions to hypoxia differed in *M. domestica* between different individuals. During the first 30 minutes of hypoxia exposure Tb changed from 33.7±0.6°C to 33.6±0.8°C in *M. domestica.* Upon initial exposure to 12-15%O_2 no apparent changes in Ta selection were observed in *M. domestica* (30.1±2.6°C to 28.8±2.6°C). However, the *P. breviceps* immediately selected higher Tas followed by lower Tas (ie 29.1°C to 34.7°C after 1 minute of hypoxia to 31.6°C after 30 minutes of hypoxia).

Qualitative observations on the effects of hypoxia on Tb indicated varying effects at different times throughout a 24-hour period. As a result, data from two time periods; 0200-0800 hours and 1200-1800 hours were analysed and compared. Significant reductions in Tb under low oxygen levels were noted from 0200-0800 hours in comparison to 1200-1800 hours for *M. domestica.* The mean core Tb of all *M. domestica* was decreased by 1.2°C during the hours 0200-0800 compared with only 0.19°C during the hours of 1200-1800 hours. For *M. domestica,* control Tb (normoxia) was significantly higher during 0200-0800 hours ($F=17.1$; $p<0.05$) however there was no significant difference between hypoxic Tb in the two time frames ($F=0.4$; $p>0.5$). Lowered oxygen levels significantly decreased Tb ($F=22.2$; $p<0.01$) during 0200-0800 time period but not during 1200-1800 hours ($F=0.7$; $p<0.5$) in *M. domestica.* This is partly due to the higher control Tb during the time period, 0200-0800 hours. Results of core Tb from individual animals during these time frames are presented in Table 8.2.4. During hypoxia, the *P. breviceps* significantly increased Tb by 0.5°C during 0200-0800 hours ($t=-16.1$; $p<0.05$) and significantly decreased Tb by 0.2°C during 1200-1800 hours ($t=6.8$; $p<0.05$). A significant difference in hypoxic Tb between the two time frames was also noted in this animal ($t=-17.5$; $p<0.05$).

Recordings of selected ambient temperatures during control and hypoxic conditions while in the thermal gradient are illustrated in Appendix 7 with a typical response for a *M. domestica* and PETAG1 illustrated in Figures 8.2.2 (a-d). The selected ambient temperatures recorded every six seconds were analysed using Fourier analysis to determine the presence or

absence of any circadian pattern of temperature selection for each animal under normal and reduced ambient oxygen levels. Data were analysed using a single series Fourier analysis as for Tb analysis. The results of this analysis are given for each animal in Appendix 12. Fourier analysis of selected Ta did not indicate the presence of a circadian rhythm in each animal in either normal or hypoxic conditions.

Table 8.2.4. Measurements of Mean Core Body Temperature (Tb) in response to Hypoxia in *Monodelphis domestica*

Tb was calculated as a mean of all Tb recordings during the six hour period
Normoxia represents 21% O_2 and Hypoxia represents 12-15% O_2
am = 0200-0800 hours pm = 1200-1800 hours
ΔTb is difference between normoxia core Tb and hypoxia core Tb
[Recordings of hypoxic core Tb are significantly different to recordings of normoxic Tb during both time periods for all animals]

Animal	Time of Day	Normoxia Core Tb (°C)	Hypoxia Core Tb (°C)	ΔTb (°C)
MOAG13	am	35.2±0.4	33.5±0.3	-1.8
MOAG13	pm	34.2±0.4	34.2±0.4	0.0
MOAG15	am	34.8±0.7	33.8±0.5	-0.9
MOAG15	pm	33.5±0.4	33.1±0.6	-0.4
MOAG16	am	34.6±0.5	34.0±0.2	-0.6
MOAG16	pm	34.3±0.2	34.2±0.2	-0.1
MOAG17	am	34.1±1.0	33.3±0.6	-0.8
MOAG17	pm	33.7±0.4	34.2±0.2	+0.5
MOAG18	am	35.6±0.4	33.7±0.4	-1.9
MOAG18	pm	34.5±0.1	33.5±0.4	-1.0
Average	*am*	*34.9±0.6*	*33.7±0.3*	*-1.2*
Average	*pm*	*34.0±0.4*	*33.8±0.5*	*-0.2*

Figure 8.2.2a Continuous Recordings of Preferred Ambient Temperature (Ta) from a *M. domestica* (MOAG18) - the effects of hypoxia
[*hypoxia begins at 12.6 hours]

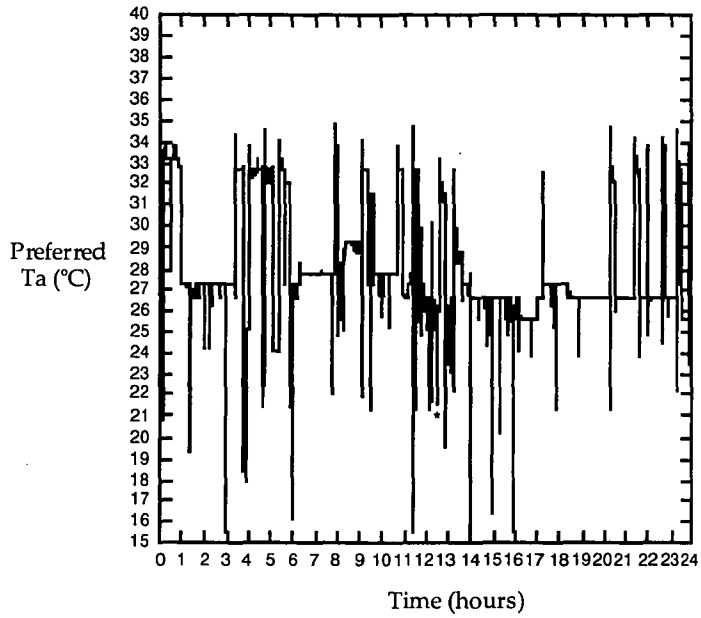


Figure 8.2.2b Continuous Recordings of Preferred Ambient Temperature (Ta) from a *M. domestica* (MOAG18) - the effects of hypoxia
[*hypoxia ends at 12.6 hours]

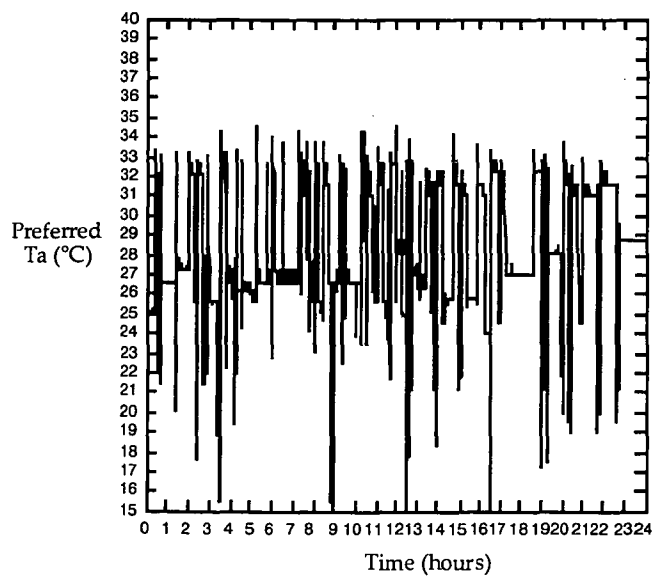


Figure 8.2.2c Continuous Recordings of Preferred Ambient Temperature (Ta) from a *P. breviceps* - the effects of hypoxia
[*hypoxia begins at 12.2 hours]

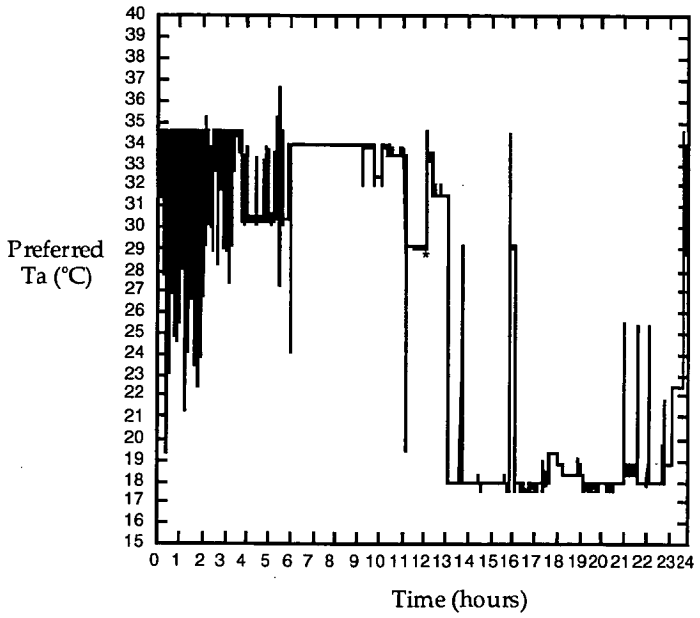
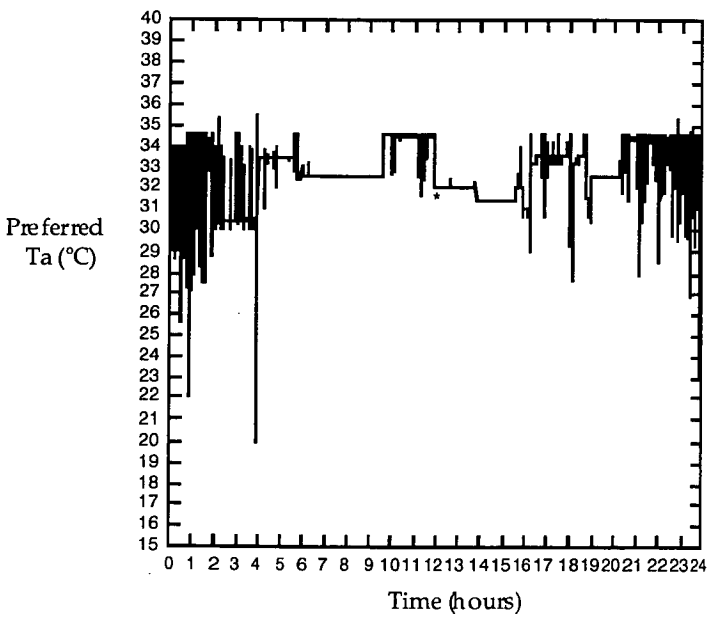


Figure 8.2.2d Continuous Recordings of Preferred Ambient Temperature (Ta) from a *P. breviceps* - the effects of hypoxia
[*hypoxia ends at 12.2 hours]



The hypoxic *P. breviceps* selected ambient temperatures around 18°C (with a high preference for 33°C also) compared to normoxic preferences of around 34°C. During hypoxia, *M. domestica* select ambient temperatures around 33°C compared to 30°C while normoxic. This is illustrated in Figures 8.2.3 (a-f) for each animal. The mean frequency of responses for selected Ta for *M. domestica* are shown in Figure 8.2.4.

Figure 8.2.3a The effect of hypoxia on the frequency of responses to ambient temperature in MOAG13

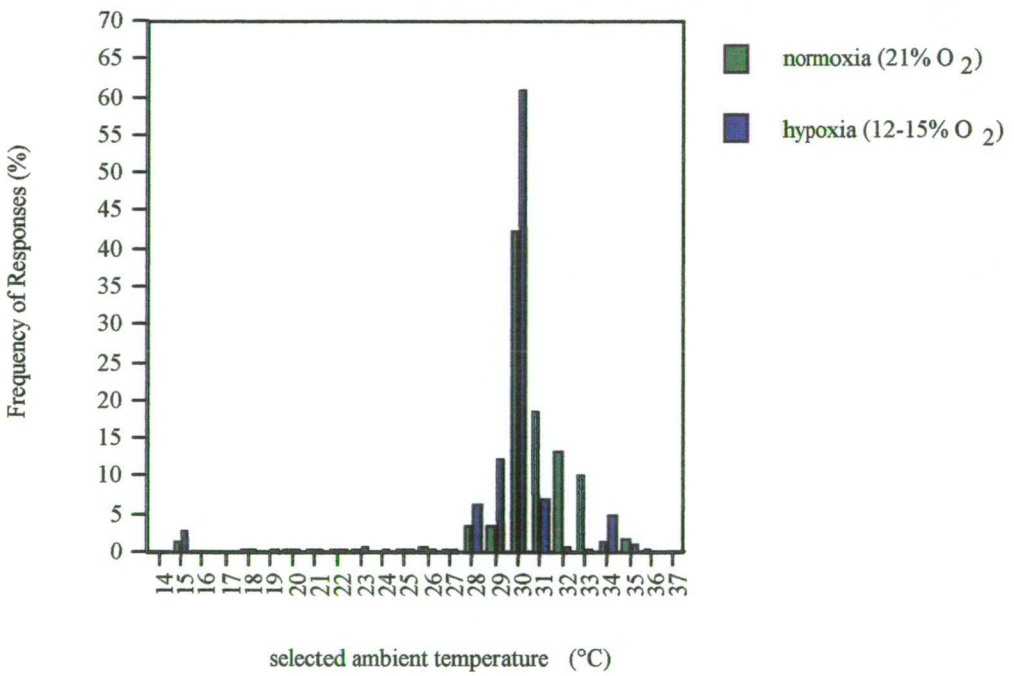


Figure 8.2.3b The effect of hypoxia on the frequency of responses to ambient temperature in MOAG15

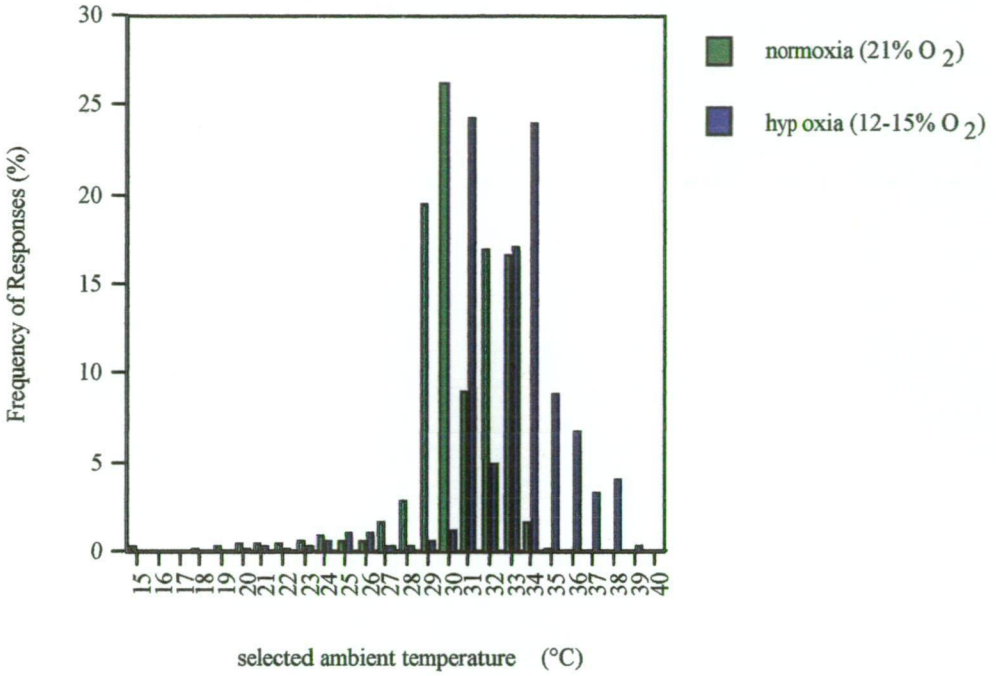


Figure 8.2.3c The effect of hypoxia on the frequency of responses to ambient temperature in MOAG16

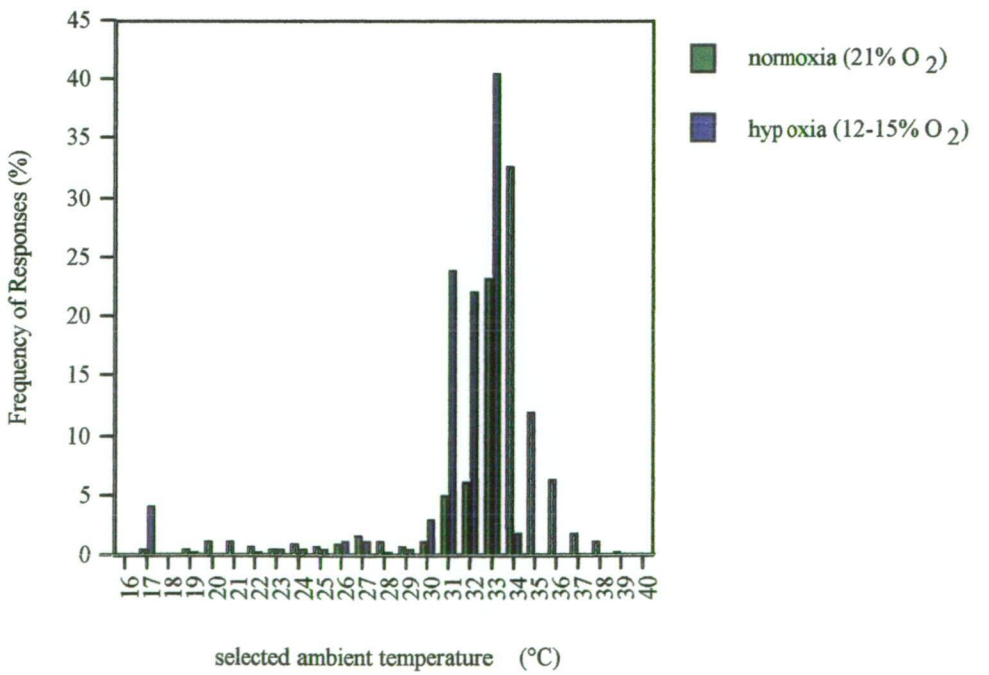


Figure 8.2.3d The effect of hypoxia on the frequency of responses to ambient temperature in MOAG17

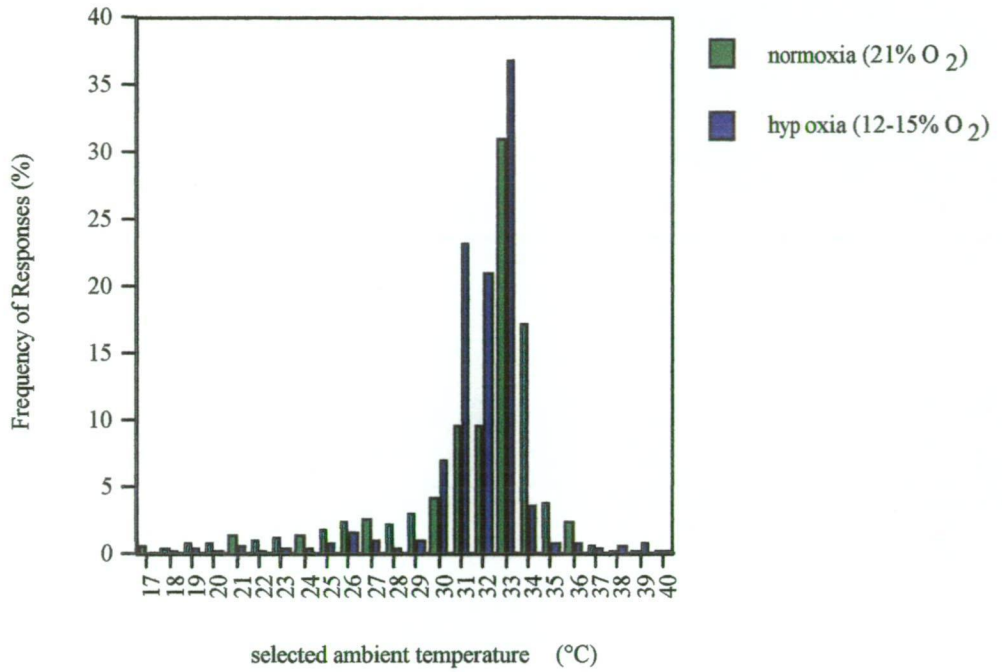


Figure 8.2.3e The effect of hypoxia on the frequency of responses to ambient temperature in MOAG18

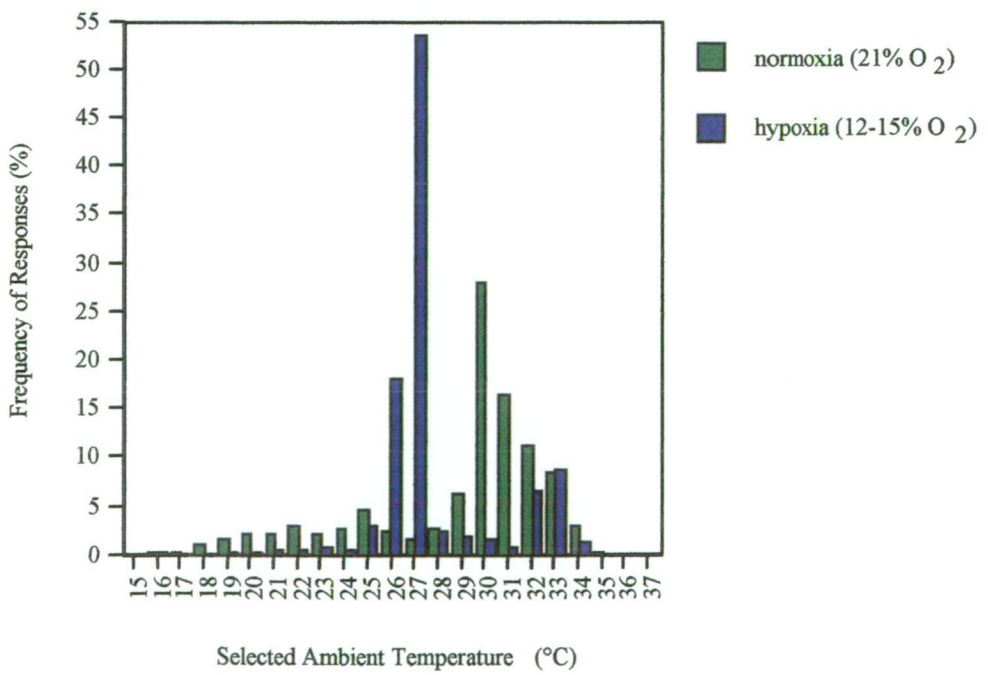
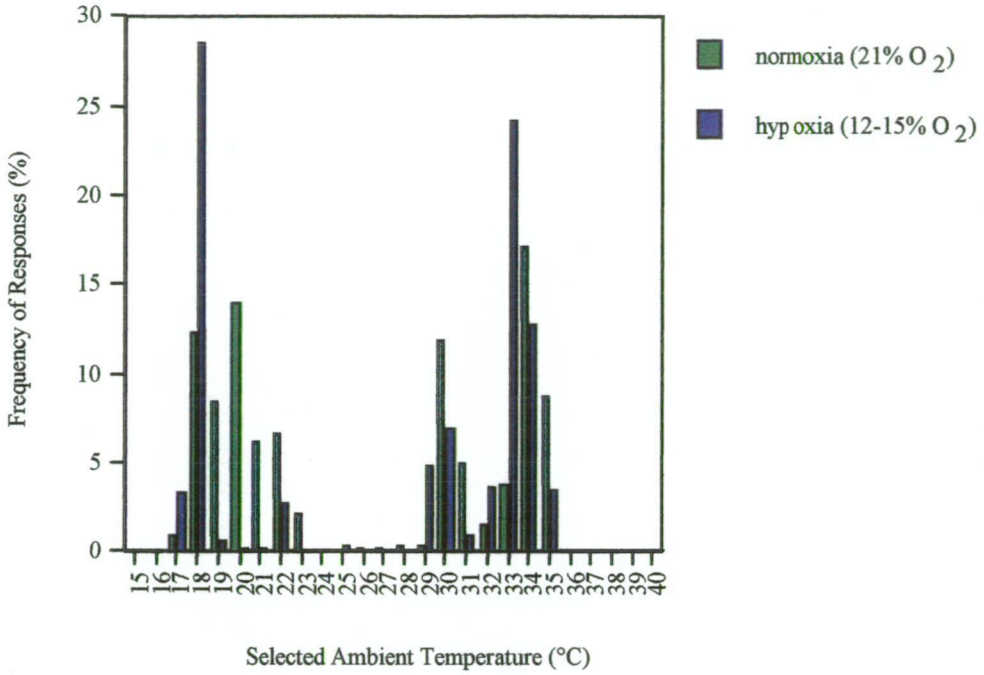
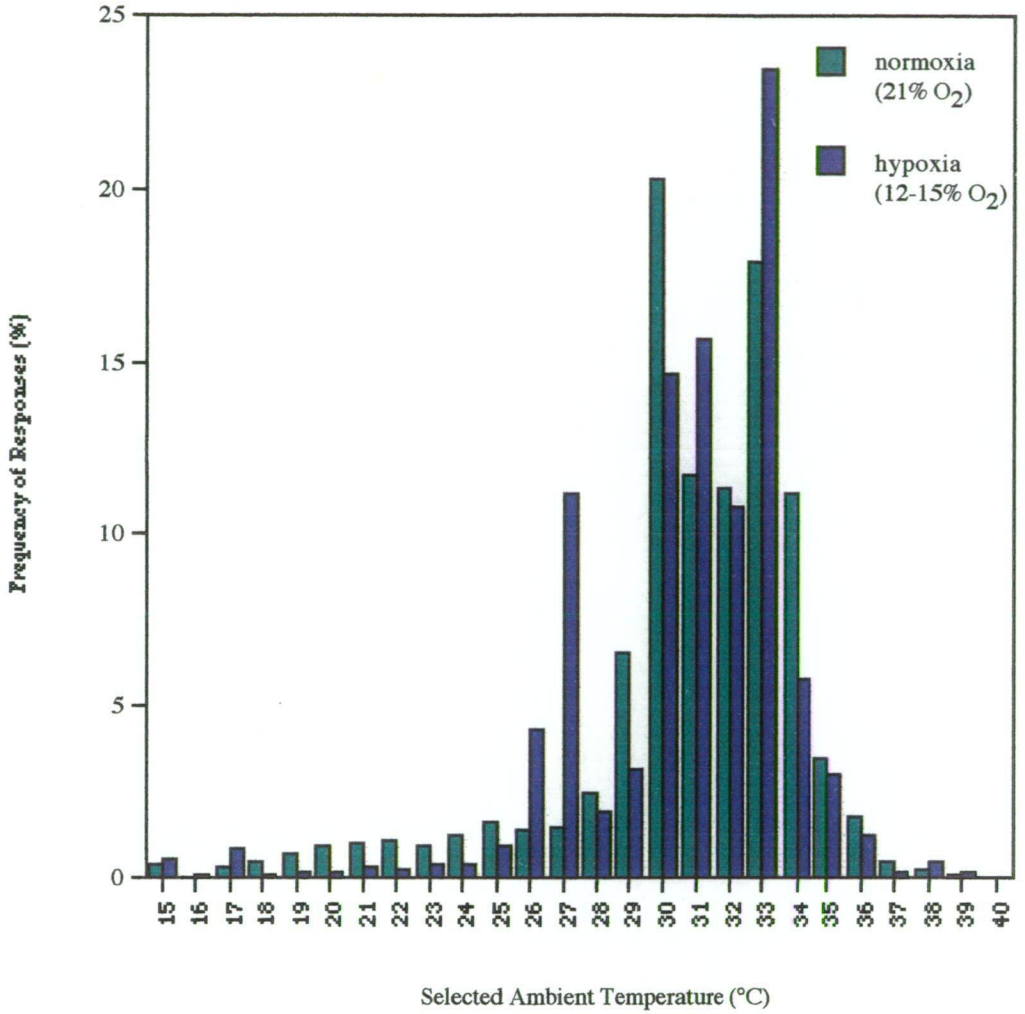


Figure 8.2.3f The effect of hypoxia on the frequency of responses to ambient temperature in PETAG1



The data presented in Table 8.2.5 indicates hypoxia did not appear to significantly affect selected Ta in *M. domestica*. Some *M. domestica* chose higher Tas in response to hypoxia while others selected lower Tas. Overall, the selected Ta under hypoxic conditions was decreased by 0.3°C during the hours of 0200-0800 and by 0.99°C during the hours of 1200-1800. Neither of these decreases were significant ($F=0.4$; $p>0.5$; $F=1.1$; $p<0.5$) and there was no significant difference between mean hypoxic Ta at each time frame ($F=0.6$; $p<0.5$) or the mean normoxic Ta at each time frame ($F=0.3$; $p>0.5$). Mean selected Tas for each *M. domestica* are presented in Table 8.2.5.

Figure 8.2.4 The effect of hypoxia on the frequency of responses to ambient temperature in *M. domestica* (n=5)



An increase in selected Ta was observed in PETAG1 during hypoxia in the 0200-0800 time period (ΔTa of 5.0°C) but this increase was not significantly different from the normoxic Ta ($t=-42.8$: $p>0.5$). There was however a significant decrease in selected Ta from 1200-1800 hours in PETAG1 ($t=133.3$; $p<0.05$) with a ΔTa of 13.1°C and mean Ta during hypoxia was significantly different between the two time periods ($t=110.5$; $p<0.05$).

Table 8.2.5. Measurements of Mean Selected Ambient Temperature (Ta) in response to Hypoxia in *Monodelphis domestica*

Selected Ta was calculated as a mean of all Ta recordings during a six hour period while the animal was in a longitudinal thermal gradient
Normoxia represents 21% O_2 and Hypoxia represents 12-15% O_2
am = 0200-0800 hours pm = 1200-1800 hours
ΔTa is difference between normoxia selected Ta and hypoxia selected Ta
[* denotes significant differences between normoxic and hypoxic selected Ta during these time periods]

Animal	Time of Day	Normoxia Selected Ta (°C)	Hypoxia Selected Ta (°C)	ΔTa (°C)
MOAG13	am*	30.7±2.4	30.4±1.0	0.3
MOAG13	pm*	30.4±1.1	29.4±1.3	1.0
MOAG15	am*	34.0±1.0	33.1±1.4	0.9
MOAG15	pm*	33.9±1.2	32.2±1.7	1.7
MOAG16	am*	32.6±1.6	31.0±4.0	2.7
MOAG16	pm*	32.2±1.1	31.3±1.1	1.0
MOAG17	am	30.1±3.0	30.9±1.3	0.8
MOAG17	pm*	29.5±4.2	31.8±1.3	2.3
MOAG18	am*	27.2±1.5	27.8±1.9	0.7
MOAG18	pm*	30.6±0.4	27.0±1.3	3.6
Average	*am*	*30.9±1.7*	*30.6±1.9*	*0.3*
Average	*pm*	*31.3±1.7*	*30.3±2.2*	*1.0*

The frequency of selected Ta at each time of the day (ie 0200-0800 and 1200-1800) was also analysed to determine the most frequented Ta within each 24 hour period. Such responses are shown in Figures 8.2.5 (a-e) and Figures 8.2.6 (a-e) for each *M. domestica*. Typically, selected Tas were distributed over a wider Ta range during 1200-1800 hours compared with 0200-0800 hours in *M. domestica* with the selected Ta being 28°C at 1200-1800 and 29°C at 0200-0800 hours during hypoxia as shown in Figures 8.2.7a and 8.2.7b. During normoxia, *M. domestica* selected a Ta that was favourably comparable to that observed during hypoxia in both time periods. During hypoxia, the *P. breviceps* selected a much lower Ta of 18°C (compared with 34°C during normoxia) between 1200-1800 hours (Figure 8.2.5f) than during 0200-0800 hours when 33°C was the selected Ta (Figure 8.2.6f). This is similar to the selected Ta of 34°C while normoxic during this time period.

Figure 8.2.5a The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in MOAG13

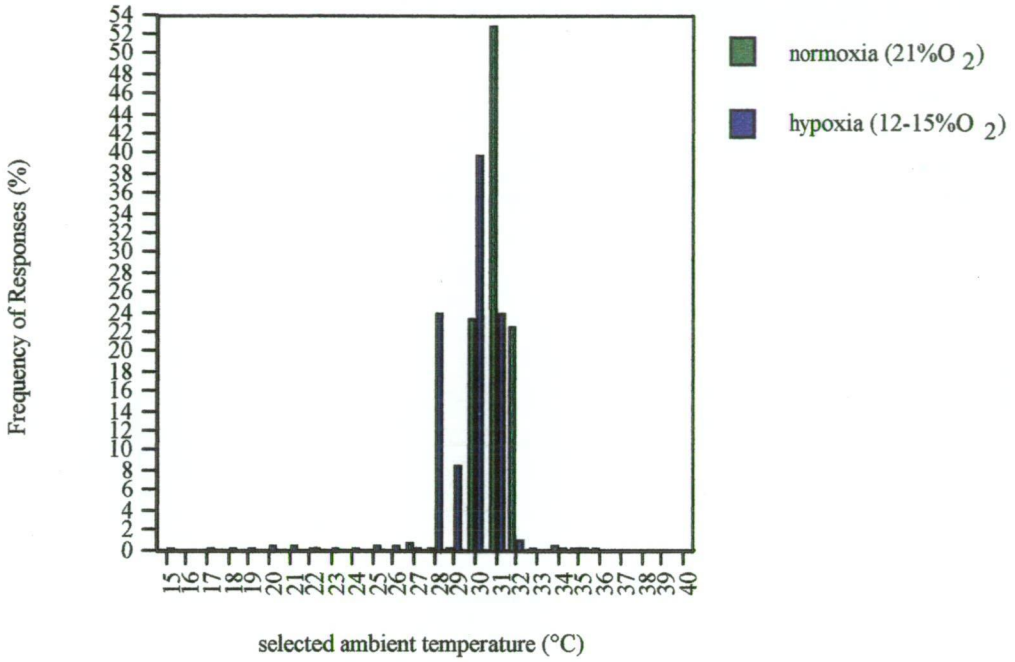


Figure 8.2.5b The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in MOAG15

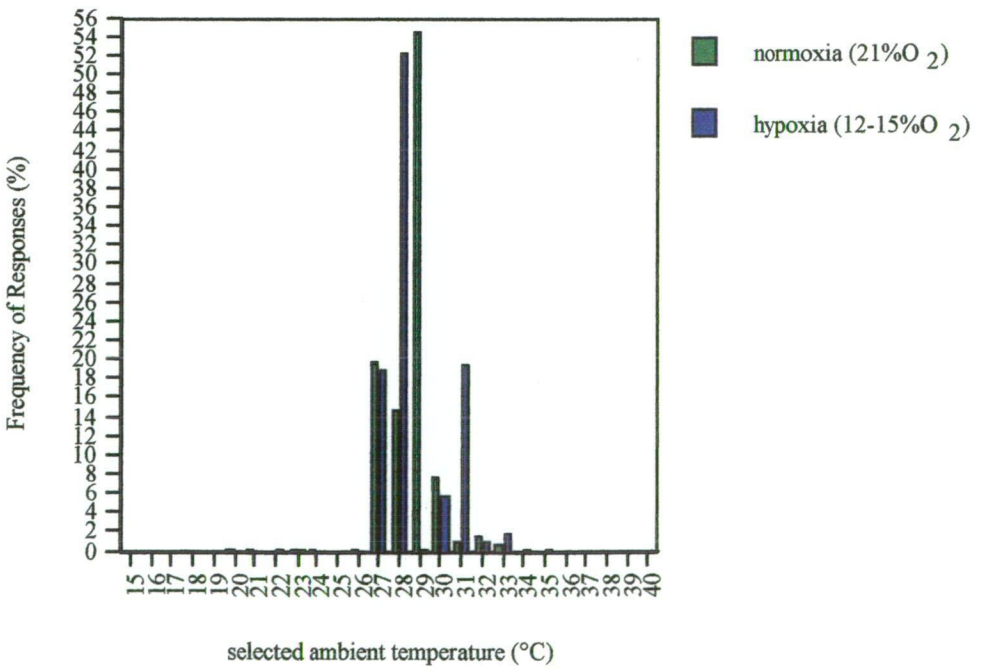


Figure 8.2.5c The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in MOAG16

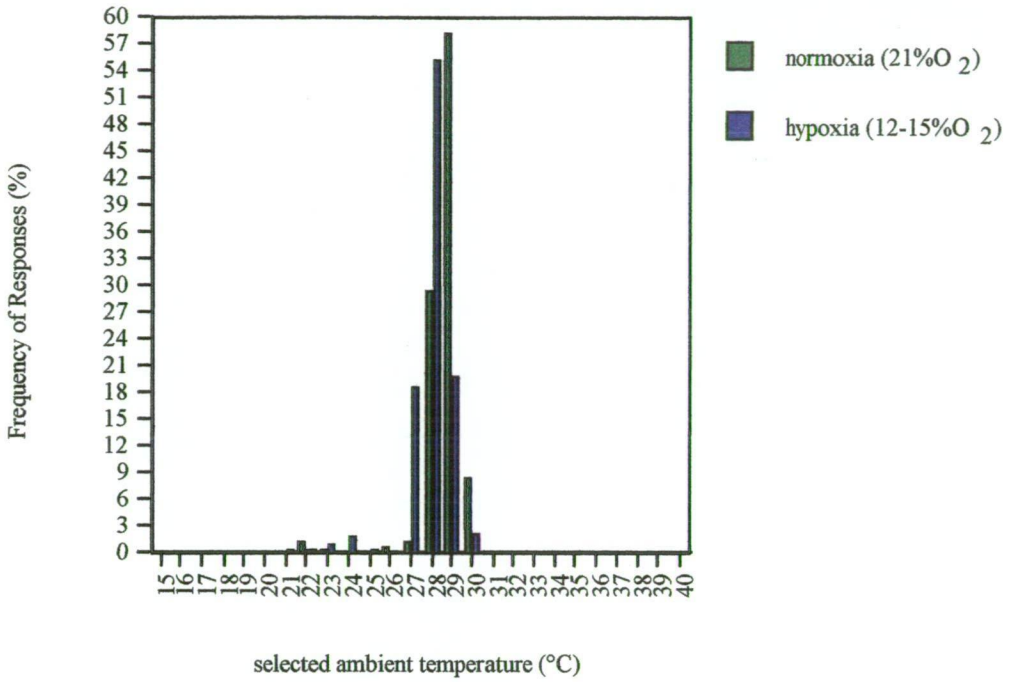


Figure 8.2.5d The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in MOAG17

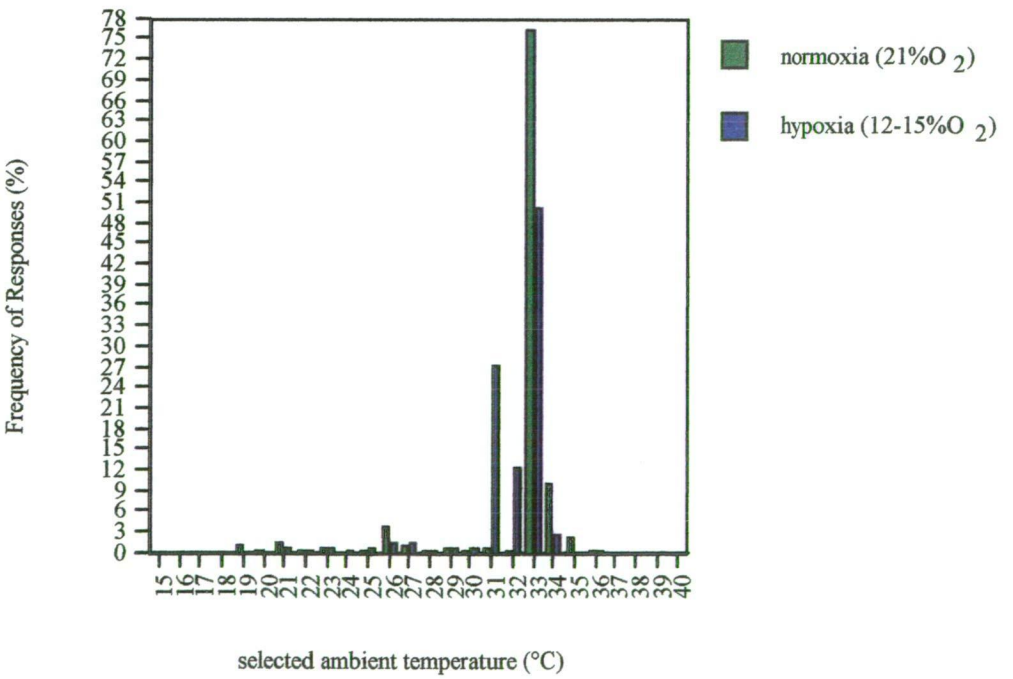


Figure 8.2.5e The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in MOAG18

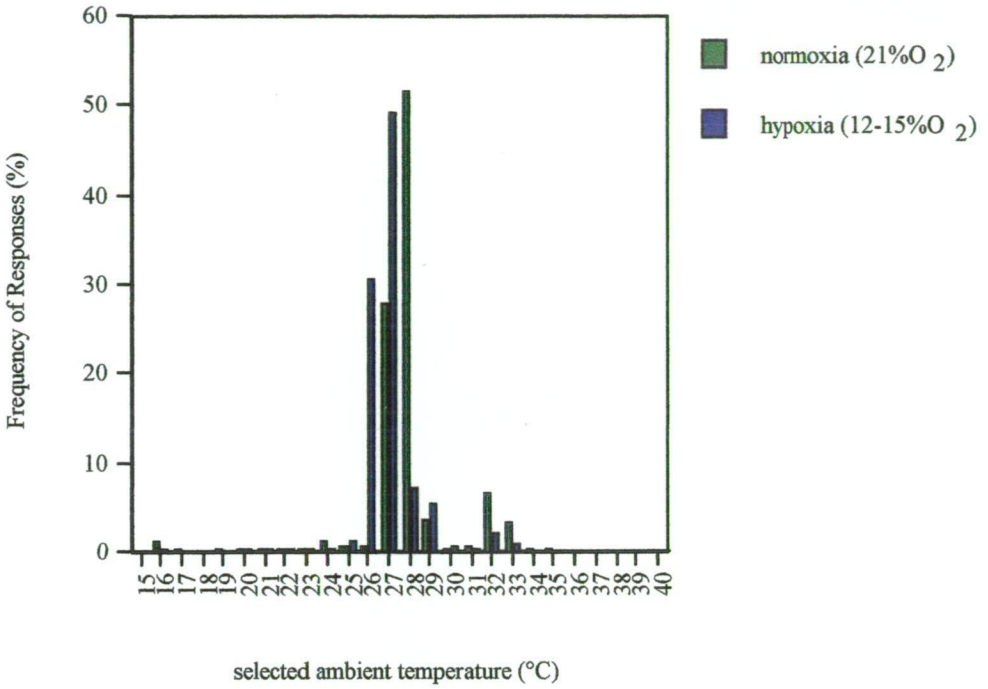


Figure 8.2.5f The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in PETAG1

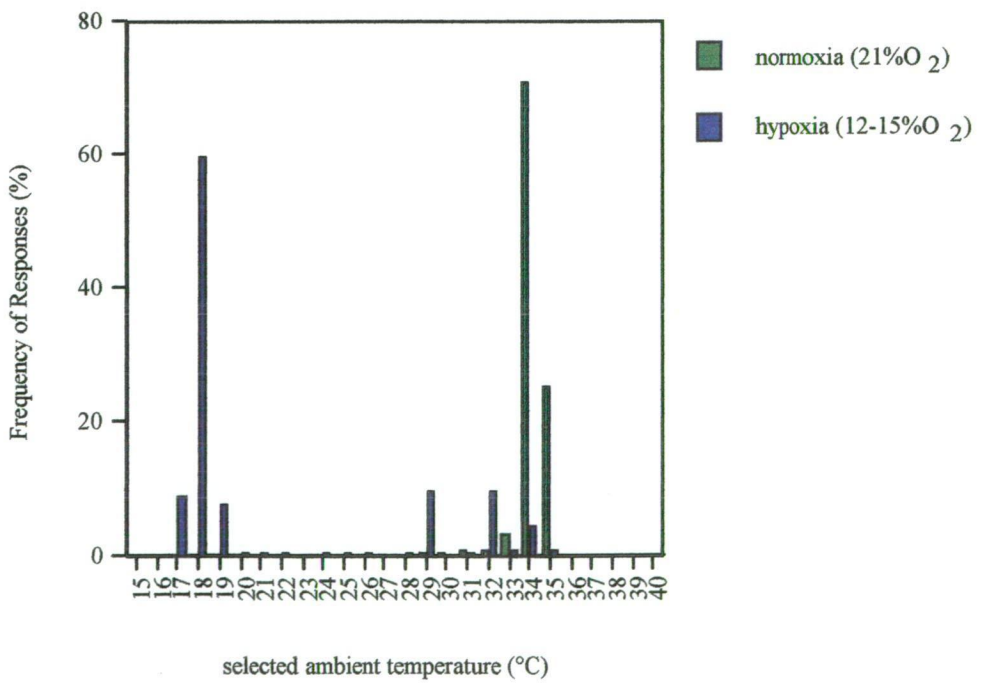


Figure 8.2.6a The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in MOAG13

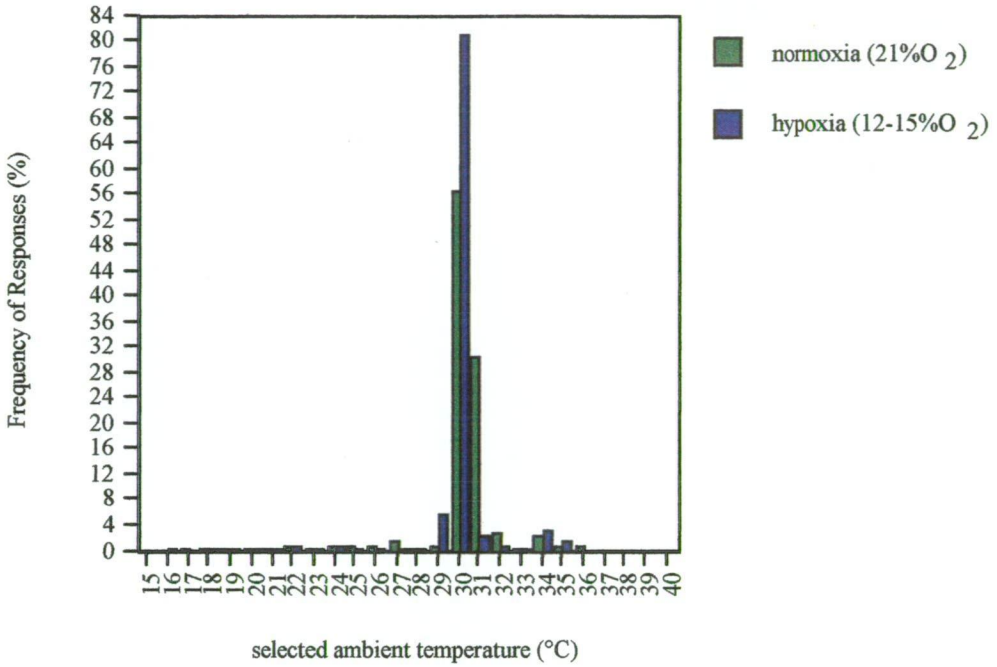


Figure 8.2.6b The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in MOAG15

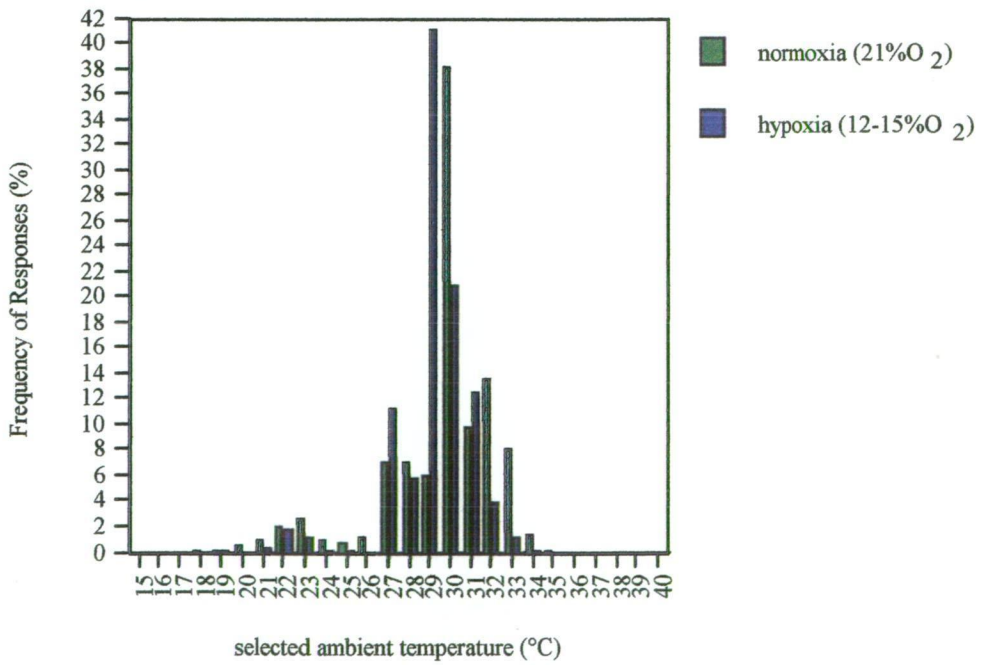


Figure 8.2.6c The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in MOAG16

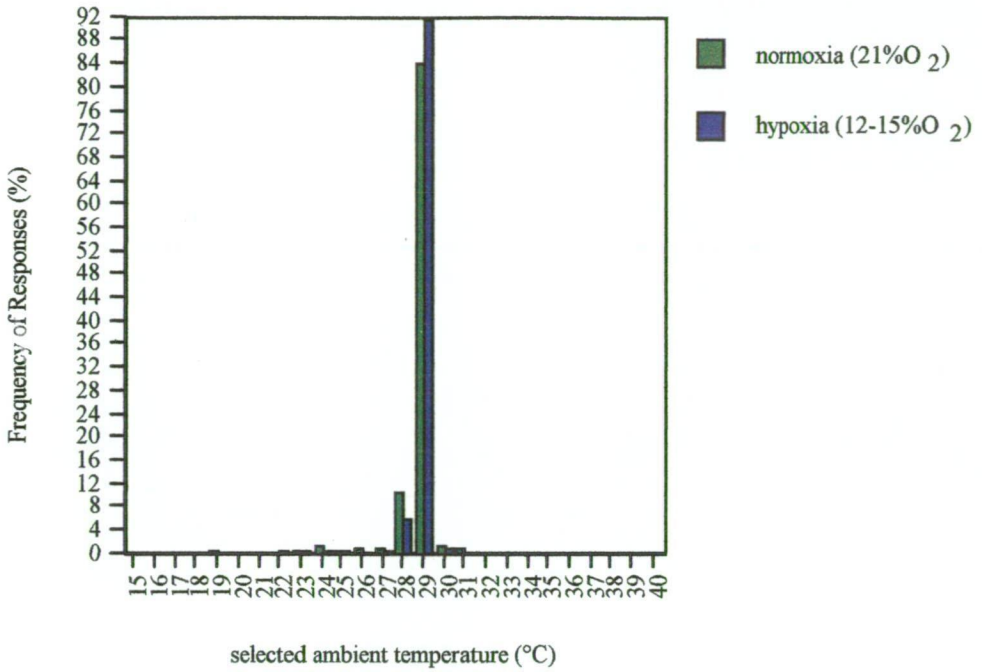


Figure 8.2.6d The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in MOAG17

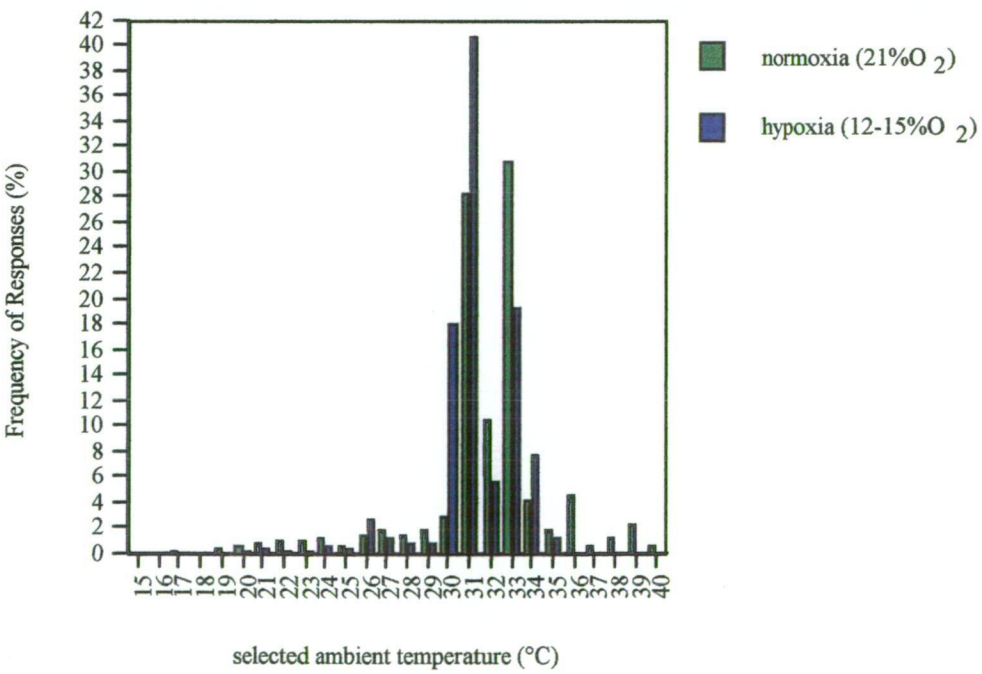


Figure 8.2.6e The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in MOAG18

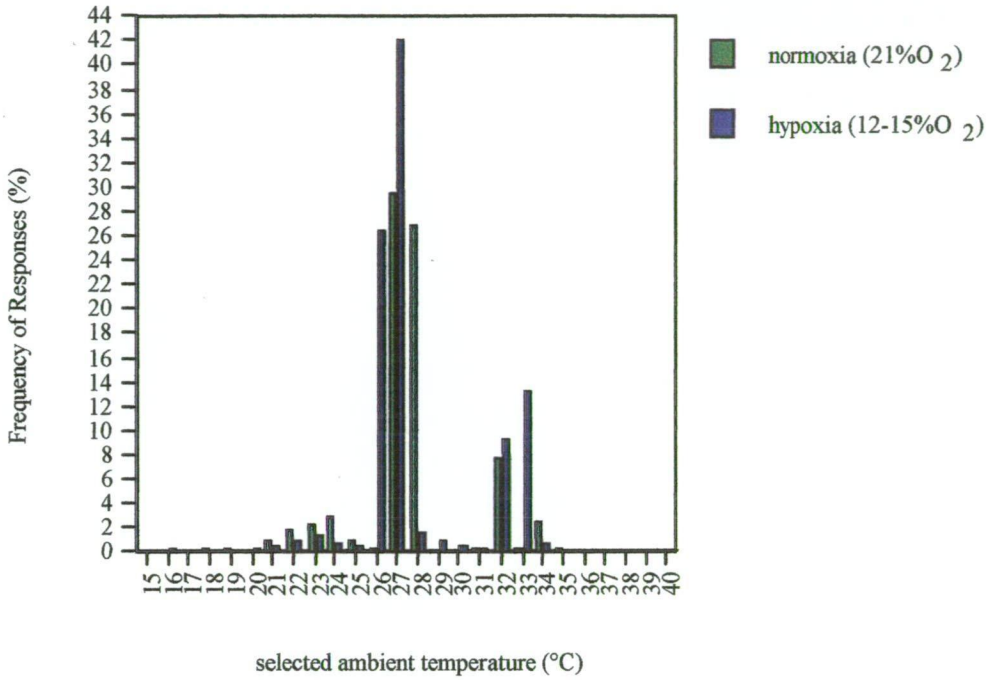


Figure 8.2.6f The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in PETAG1

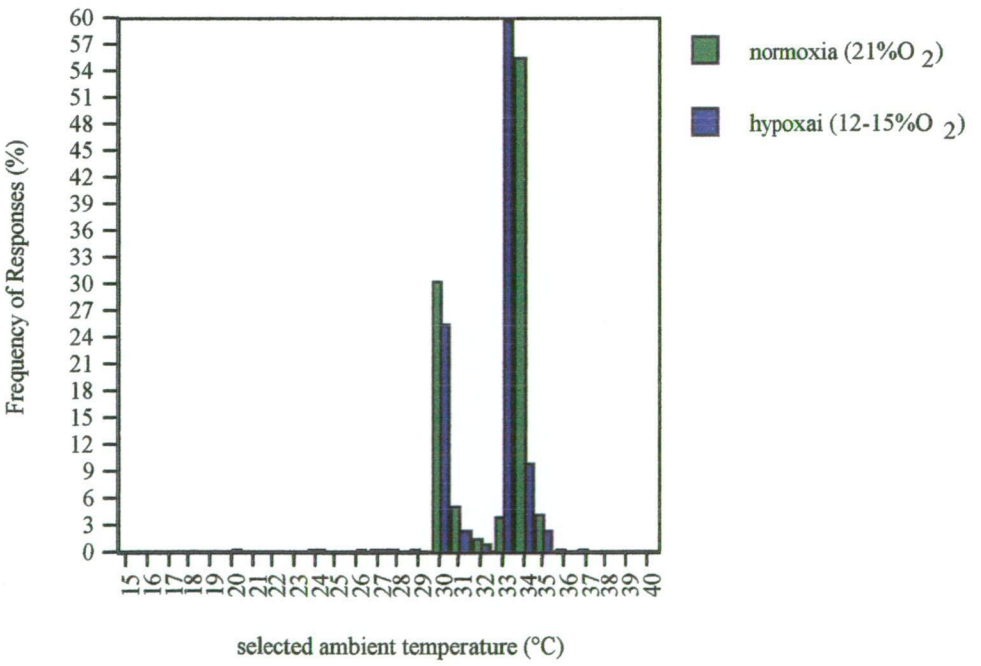


Figure 8.2.7a The effect of hypoxia on the frequency of responses to ambient temperature during the time period 1200-1800 hours in *M. domestica* (n=5)

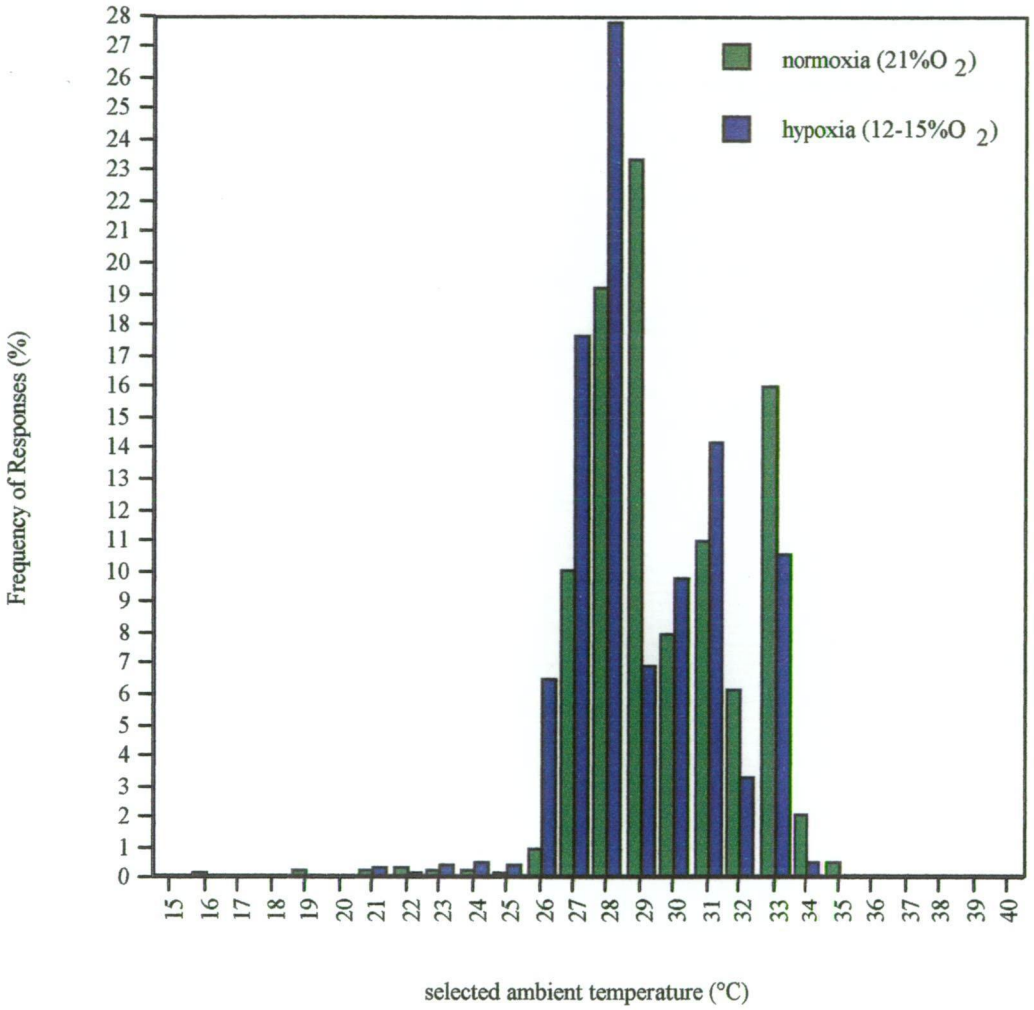
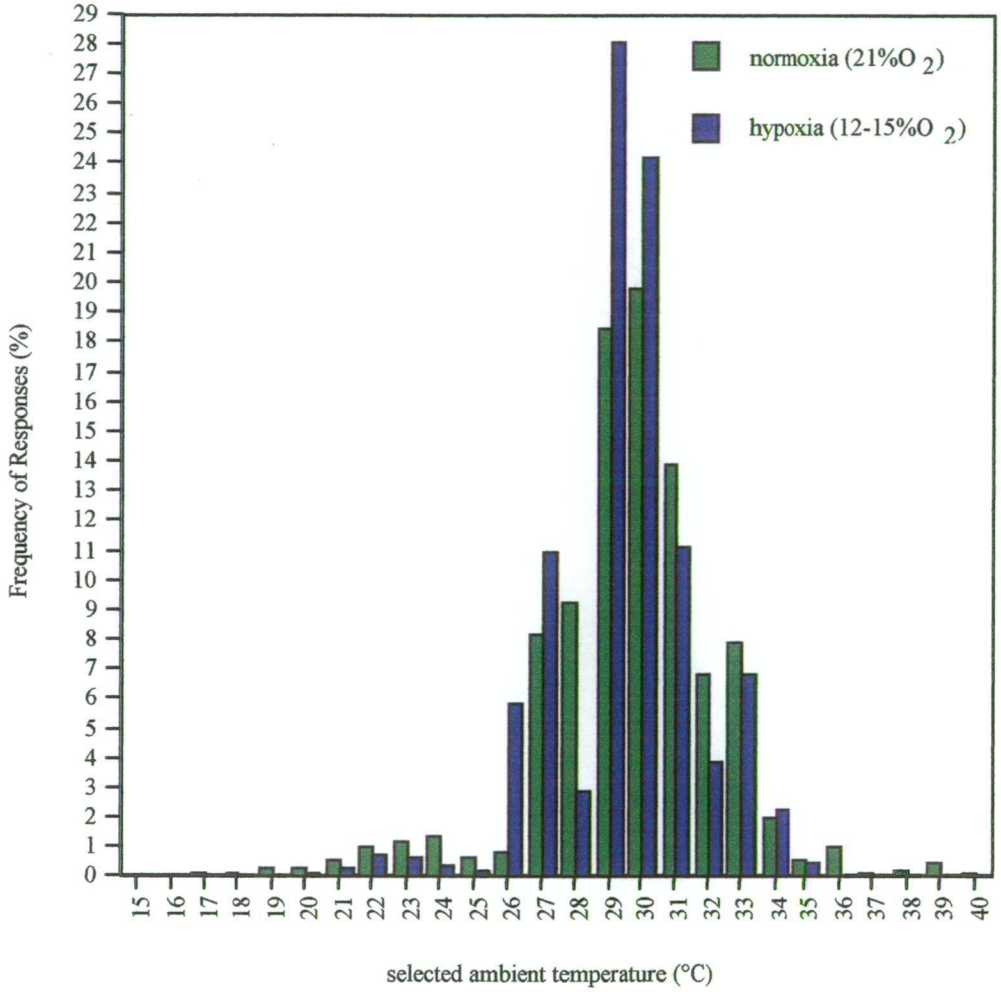


Figure 8.2.7b The effect of hypoxia on the frequency of responses to ambient temperature during the time period 0200-0800 hours in *M. domestica* (n=5)



8.2.2 Effects of Hypoxia on Metabolic Rates in *M. domestica*

Basal metabolic rates were measured in five *M. domestica* over a 24-hour period as shown in Table 8.2.6. Similar rates were noted for each animal with an average of 0.6ml/g/hr.

Table 8.2.6 Basal Metabolic Rates (BMR) in *Monodelphis domestica*

Animal	Mass (g)	BMR (ml/g/hr)
MOAG13	118	0.6±0.2
MOAG14	100	0.5±0.2
MOAG15	126	0.5±0.1
MOAG16	114	0.6±0.2
MOAG17	132	0.6±0.2
Average	*118±12.2*	*0.55±0*

Metabolic rates were also measured in each animal at Tas of 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C during control conditions (21% O_2) and hypoxic conditions (12-15% O_2). Metabolism was significantly decreased in hypoxic conditions compared to normoxic conditions ($F=205.8$; $p<0.001$) at all Tas. Metabolic rates are shown in Table 8.2.7 and illustrated in Figure 8.2.8 and Figure 8.2.9. Metabolic rates of individual animals were more variant at 35°C during normoxia yet this high level of variance is also apparent at 40°C during hypoxia. Figure 8.2.10 shows the reduced levels of metabolic rate for *M. domestica* during hypoxia compared to normoxia at each of the exposed Tas. The TNZ is observed to be somewhere between 30°C and 40°C during both normoxia and hypoxia in *M. domestica.*

Figure 8.2.8 Levels of Metabolism at various Ambient Temperatures in *M. domestica*

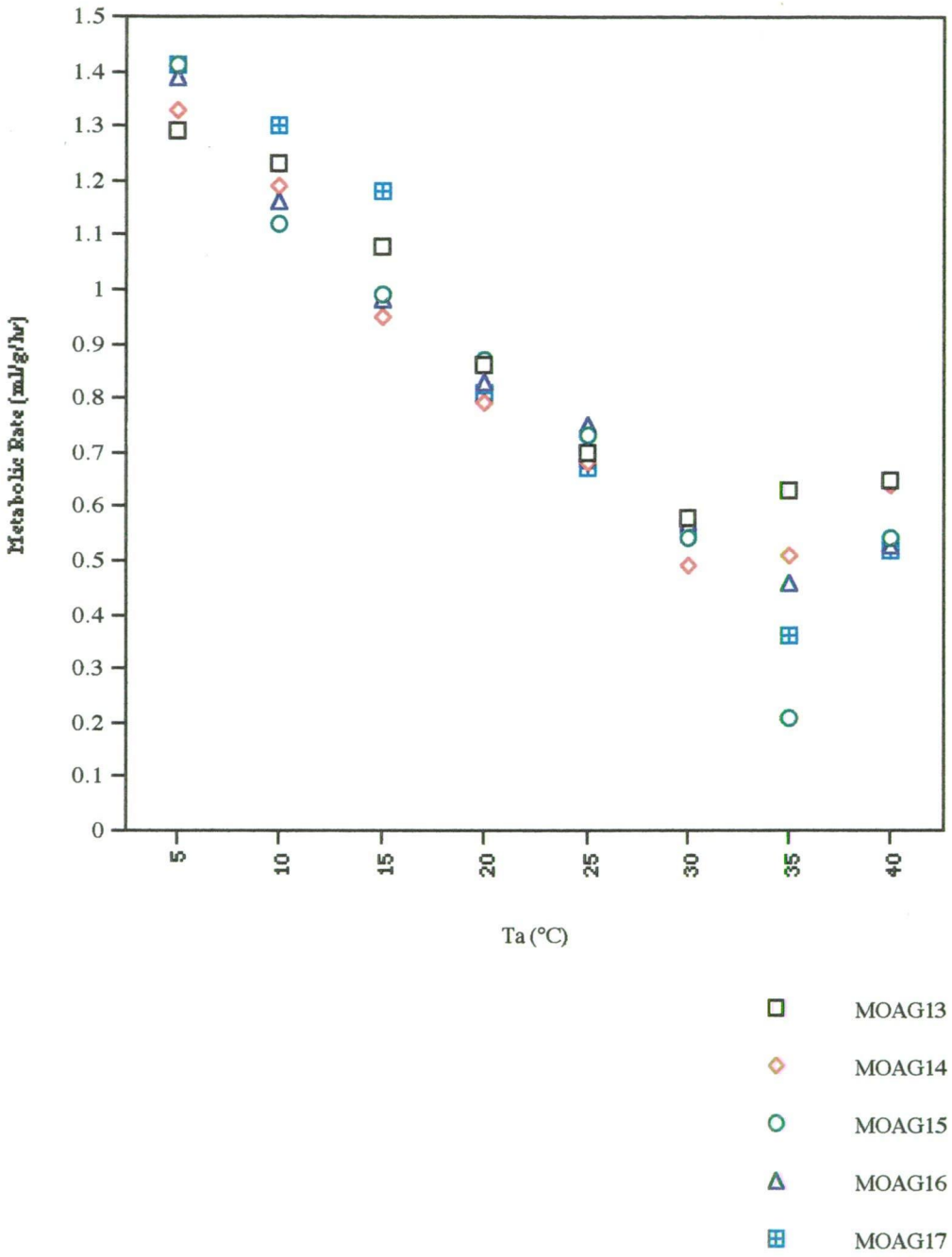


Figure 8.2.9 Levels of Metabolism during Hypoxia in *M. domestica* at various Ambient Temperatures

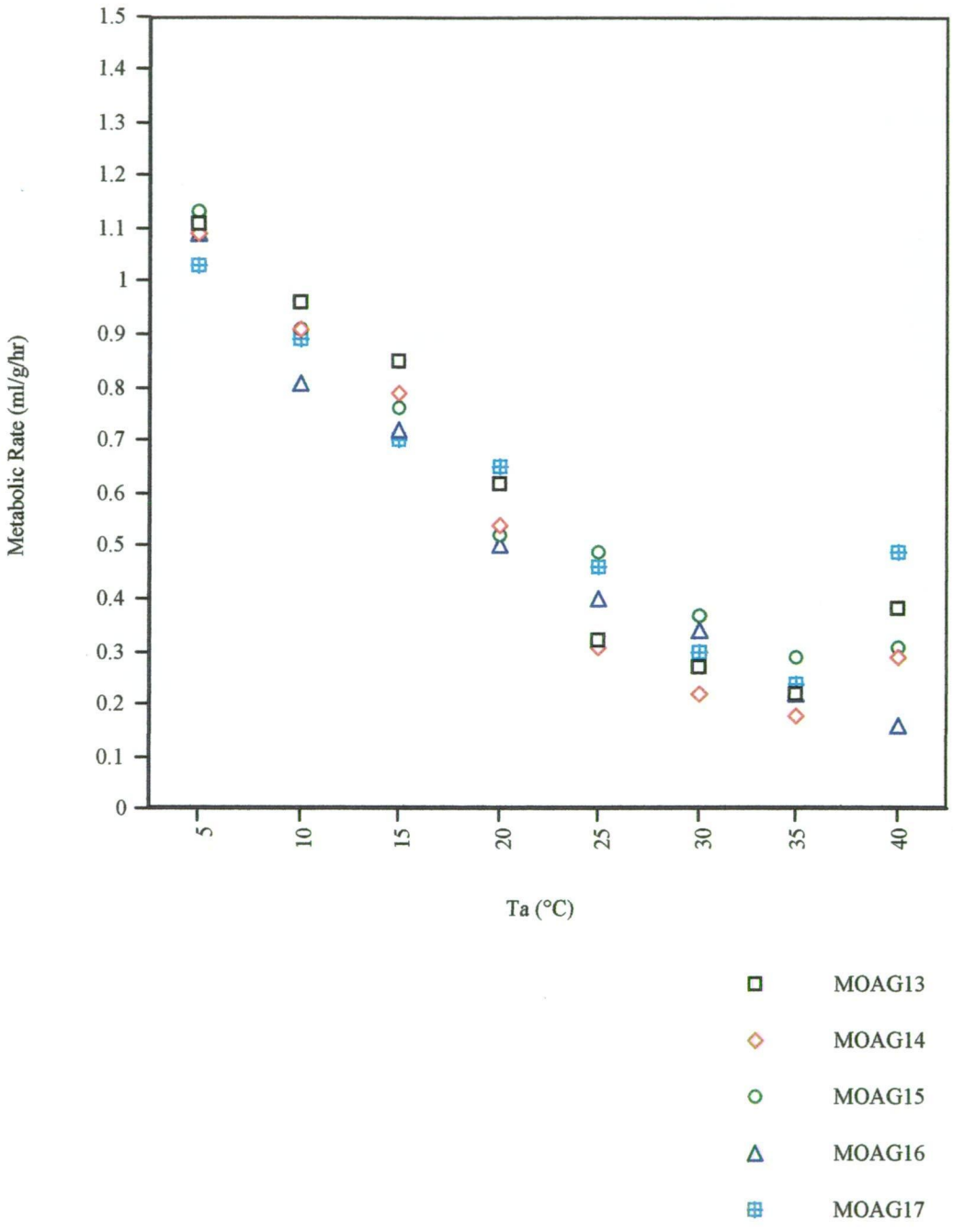
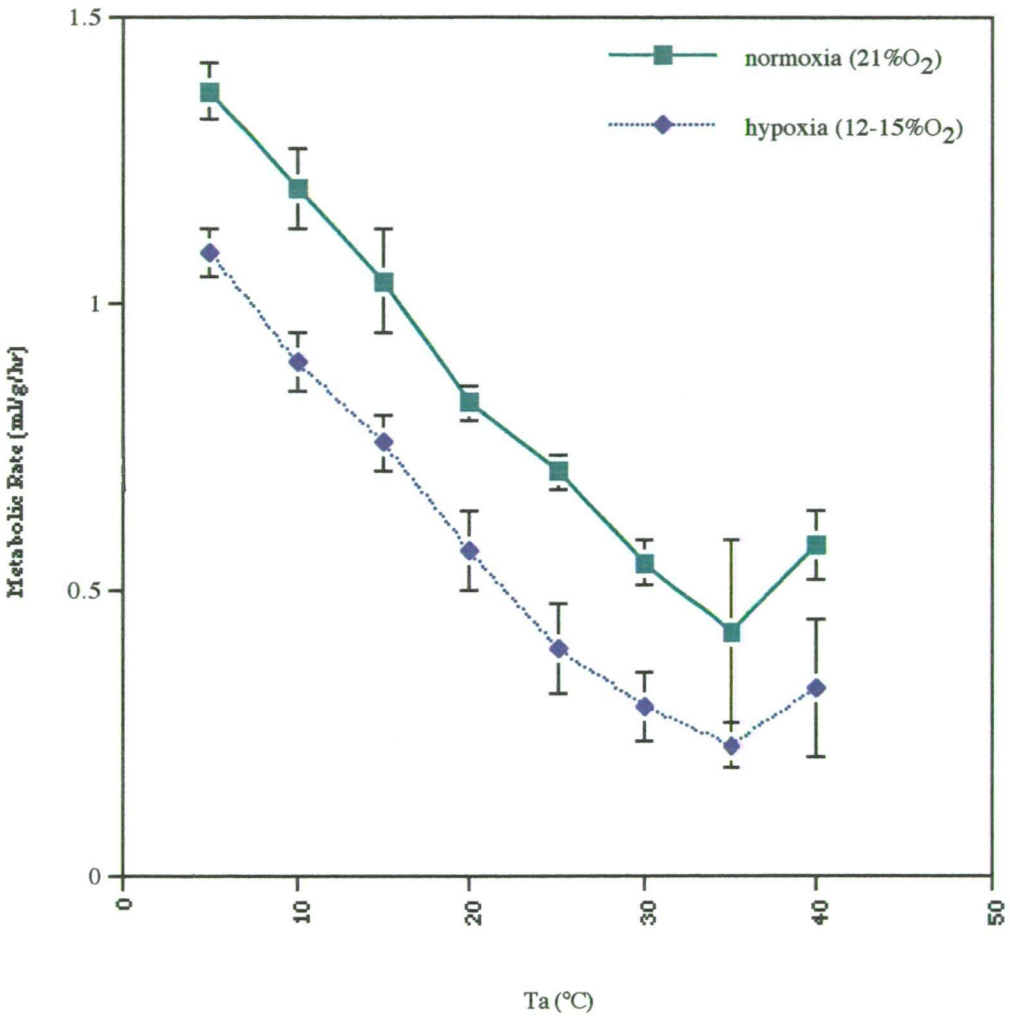


Table 8.2.7 Metabolic Rates (MR) in *Monodelphis domestica* at different Ambient Temperatures (Ta) during Normoxia and Hypoxia

Normoxia represents 21% O_2 and Hypoxia represents 12-15% O_2
Metabolic rates at hypoxia were significantly different from metabolic rates at normoxia for all animals.

Animal	Ta (°C)	MR at normoxia (ml/g/hr)	MR at hypoxia (ml/g/hr)
MOAG13	5	1.3	1.1
	10	1.2	1.0
	15	1.1	0.9
	20	0.9	0.6
	25	0.7	0.3
	30	0.6	0.3
	35	0.6	0.2
	40	0.7	0.4
MOAG14	5	1.3	1.1
	10	1.2	0.9
	15	1.0	0.8
	20	0.8	0.5
	25	0.7	0.3
	30	0.5	0.2
	35	0.5	0.2
	40	0.6	0.3
MOAG15	5	1.4	1.1
	10	1.1	0.9
	15	1.0	0.8
	20	0.9	0.5
	25	0.7	0.5
	30	0.5	0.4
	35	0.2	0.3
	40	0.5	0.3
MOAG16	5	1.4	1.10
	10	1.2	0.8
	15	1.0	0.7
	20	0.8	0.5
	25	0.8	0.4
	30	0.6	0.3
	35	0.5	0.2
	40	0.5	0.2
MOAG17	5	1.4	1.0
	10	1.3	0.9
	15	1.2	0.7
	20	0.8	0.7
	25	0.7	0.5
	30	0.6	0.3
	35	0.4	0.2
	40	0.5	0.5
Average	*5*	*1.4±0.1*	*1.1±0.0*
	10	*1.2±0.1*	*0.9±0.1*
	15	*1.0±0.1*	*0.8±0.1*
	20	*0.8±0.0*	*0.6±0.1*
	25	*0.7±0.0*	*0.4±0.1*
	30	*0.6±0.0*	*0.3±0.1*
	35	*0.4±0.2*	*0.2±0.0*
	40	*0.6±0.1*	*0.3±0.1*

Figure 8.2.10 Metabolic Rates at 5*C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°c during normoxia and hypoxia in *M. domestica* (n=5)



8.3 Discussion

As hypothesised, during hypoxia, a reduction in core Tb is apparent in *M. domestica* while held in a temperature gradient ranging from approximately 12°C to 40°C. This is supported by previous studies in which body temperatures have been found to be significantly reduced by low levels of ambient oxygen in reptilian and eutherian species (eg Wood and Gonzales, 1996). In this study, a drop in Tb occurs in association with a significant change in the normal selected Ta particularly over a circadian cycle. The responses to Ta vary however among individual animals with a dramatic reduction in selected Ta observed in the *P. breviceps* during the hours of exposure to hypoxia. Such a response was not apparent in any *M. domestica*. Reduced levels of metabolism were also observed in *M. domestica* at various Ta during hypoxia as previously seen in marsupials and other mammals (eg Frappell et al., 1992). This hypometabolic state during hypoxia indicates a decrease in oxygen use by hypoxic tissues as the animal adjusts tissue metabolism to the reduced oxygen availability.

8.3.1 Effects of Hypoxia on Core Body Temperature

Exposure to hypoxia for 24 hours resulted in a reduced core Tb in *M. domestica* which was maintained until ambient O_2 levels returned to 21%. Unlike previous observations, an acute decrease in Tb was not always immediately observed in *M. domestica* upon exposure to hypoxia. Two explanations may account for this. Firstly, the level of hypoxia was not particularly low at 12-15% O_2 therefore thermogenic adjustments would not be as pronounced as this level of hypoxia would not be extremely limiting with respect to energy requirements. Secondly, the animals were in a thermal gradient unlike most other studies of hypoxic effects on Tb which have been performed at room temperature. This enabled them to choose a preferred Ta while hypoxic and even though no obvious patterns of Ta selection are observed in this species during hypoxia over a 24-hour period, it may be that the Ta at which they were located when hypoxia exposure

began was within their preferred range for hypothermia. This is highly likely given that *M. domestica* tended to choose Tas only slightly lower than normal while in a hypoxic environment.

Frappell et al (1992) found that exposure to hypoxia at 10% for 10-20 minutes in *P. breviceps* results in a reduction in core Tb of 1.6°. However, this study observed a decrease of only 0.5°C within the first 20 minutes of hypoxia despite an average decrease of 0.9°C over a 24-hour period for this species. This may be accounted for by the different methods of Tb recording (ie rectal probe vs implanted radiotransmitter) or the higher level of ambient $\%O_2$ used in the current study. According to Frappell et al., (1992), marsupials decrease thermogenesis and metabolic rate in response to hypoxia with decreases in Tb as great as 2.4°C in *Sminthopsis crassicaudata* and as little as 0.3°C in rat kangaroos such as *P. tridactylus* and *B.penicillata*. The results of this study fit within this range with 24-hour exposures to hypoxia resulting in a mean decrease of 0.47°C and 0.89°C in *M. domestica* and the *P. breviceps* respectively.

Hypothermia is a useful response to hypoxia as hypoxia results in the absence of a normal thermogenic response except in hypoxia-tolerant tissues such as skeletal muscle and brown fat (Wood and Gonzales, 1996). Hypoxia induces hypothermia by decreasing heat production and increasing heat loss in a variety of eutherians (eg Gellhorn and Janus, 1936; Horstamn and Banderet, 1977; Gautier et al., 1987; Dupré et al., 1988; Gautier et al., 1991; Gautier, 1996). Decreased heat production is partly due to the redistribution of blood flow away from brown fat in rats (Szenelyi and Donhoffer, 1968) and selecting a lower Ta will increase the rate of heat loss. This is advantageous as it lowers metabolic rate and thus oxygen requirements thereby enhancing survival. The potential benefits of hypothermia in hypoxic animals include survival (particularly in neonates), brain cooling thus preventing potential cell damage, a reduction in resting tissue metabolism, reduced ventilation and alterations in O_2 transport resulting in improved O_2 loading in the lungs (Wood and Malvin, 1992). Conversely, too

low a Tb could be disadvantageous affecting normal brain development and immune responses, metabolic rate during sympathetic responses and causing ventricular fibrillation (Wood and Malvin, 1992) and in severe cases progress to hypothermic death. Therefore, there must be a delicate balance between energy savings and the level of Tb reduction. This may be modulated by thermoregulatory behaviour as shown in the *P.breviceps* in this study.

8.3.2 Effects of Hypoxia on Selected Ambient Temperature over a 24-hour period

During hypothermia one might expect animals to preferentially choose warmer environments. A preference for a cooler environment suggests that as a result of some physiological readjustment (ie a change in hypothalamic set-point, vasomotor activity or metabolism) the "set" thermal comfort zone has also been temporarily altered. It has been previously shown that rodents select lower Tas when hypoxic supporting the hypothesis that hypoxia lowers the thermoregulatory set-point in these mammals (Gordon and Fogelson, 1991). This was also readily observed in a study by Gumma and South (1970) who found that after mild hypothermia hamsters select cooler temperatures before returning to their normal preference for a warmer Ta. Similarly, Clark and Fewell (1996) reported a decrease in selected Ta in newborn guinea pigs exposed to hypoxia with relatively no change in selected Ta in adults under the same conditions. Dupré and Owen (1992) however found that the rat selects a significantly higher Ta when exhibiting a hypothermic response under hypoxia. This latter observation may be related to the animal preventing a dramatic fall in Tb by selecting a Ta above the TNZ. Cold exposure could exaggerate the hypothermic response to hypoxia by eliciting a thermogenic response (ie shivering and NST) and increasing O_2 demand.

In this study, mean selected Ta over a 24-hour period was either decreased or increased in *M. domestica* although all changes were insignificant. By contrast, the hypoxic *P. breviceps* showed a marked

decrease in selected Ta (after initial selection of a warmer Ta) with great variance in temperature selection particularly during the light phase. This indicates that marsupials are not immediately seeking cooler environments during hypoxia but instead are utilising their environment to minimise the extent of hypothermia. This would be due to the absence of thermogenesis during hypoxia as observed in rats while in cold Ta (Dupré et al., 1988).

Although, over a 24-hour period of hypoxia, the *P. breviceps* selected significant lower Tas than while normoxic the same did not hold true when considering the initial behavioural response to the lower ambient O_2 level. During the first 30 minutes of hypoxia a dramatic increase in selected Ta occurred in this animal while Tb simultaneously decreased. This indicates that initially the animal limited the reduction in Tb by selecting a warmer Ta and may be viewed as an energetically efficient mechanism of Tb regulation when hypothermic. This has similarly been seen in toads at 12% O_2 (Wood and Malvin, 1991) rats at 10.2% O_2 (Dupré and Owen, 1992), mice at 11.5% O_2 (Gordon and Fogelson, 1991), hamsters at 14.7% O_2 (Gordon and Fogelson, 1991). This hypothermia-limiting effect through behaviour may explain why the hypoxic hypothermia in the *P. breviceps* in this study was not as dramatic as that observed by Frappell et al., (1992). After an initial selection of warmer Tas, the *P. breviceps* then selected significantly colder Tas while still hypoxic particularly during the 1200-1800 time period.

The effects of short-term hypoxia on *M. domestica* were inconclusive although one animal did show an immediate and dramatic decrease in Tb upon exposure to hypoxia. The effects of hypoxia on Ta selection during this initial period however, were minimal in this species. As these animals were laboratory bred their constant (elevated) Ta environment from birth may have affected their hypoxic drive. Hypoxia affected Tb in *M. domestica* similarly throughout the 24-hour period and although Tas selected during hypoxia were lower they were not significant.

Reductions in selected Ta with simultaneous decreases in core Tb have often been observed in rodents over short time periods. Acute levels of hypoxia administered to mice, hamsters and rats for 60 minutes lowers Tb dramatically with selected Ta decreasing when O_2 levels were particularly low at 6-7% (Gordon and Fogelson, 1991). At ambient O_2levels of 10-13% Gordon and Fogelson (1991) found that rodents selected Tas similar to that selected during normoxia. This is comparable to the present study in which hypoxia levels were equivalent to 12-15% O_2. It seems apparent from these studies that acute hypoxia at low ambient O_2 levels (ie below 10% O_2) may result in a change in the thermal set-point while hypoxia above this critical level results in an increase or no change in Ta preference indicating no change in the set-point of thermoregulation.

The level of sensitivity to ambient O_2 levels may differ between different species and different individuals resulting in relative higher or lower critical values. In this study, the *P. breviceps* indicated a higher sensitivity to O_2 levels than *M. domestica*. This sensitivity was also seen by Frappell et al., (1992) who found that *P. breviceps* had a significant decrease in Tb when exposed to 10% O_2 for 20 minutes. The threshold O_2 level for behavioural hypothermia during hypoxia has previously been found to be different in various species (both ectothermic and endothermic) due to their varying degrees of hypoxic tolerance (Wood and Gonzales, 1996). However a common threshold for a behavioural response to hypoxia is 10% O_2 in various taxa (Wood and Malvin, 1992). This threshold value presumably relates to the oxyhaemoglobin dissociation curve as 10% O_2 will produce marked desaturation of haemoglobin in arterial blood. This common threshold may exist for *M. domestica* but on the current evidence it appears that *P. breviceps* has a higher threshold for hypoxia. This may be related to the altitudes of the natural habitats of these species. Thermoregulatory behaviour was affected by hypoxia in the *P. breviceps* but not in *M. domestica*.

In the absence of thermoregulatory behaviour, Tb can still be regulated by insulative, evaporative and metabolic adjustments. It has been

shown by Dupré and Owen (1989) that the effects of hypoxia on Tb in rats is the same in the presence and absence of a thermal gradient. This suggests the relative unimportance of the environment with respect to the maintenance of hypothermia during hypoxia. However, as pointed out by Dupré and Owen (1989), it is possible that the constant Ta used during the experiments lacking the thermal gradient may be within the range which would be selected by the animal while hypoxic. This may also apply to the observations from this study in that the animals selection of Ta during normoxia suited thermogenic requirements during hypoxia at most time periods within 24 hours. Differences in temperature selection at different time periods by individual animals also supports this observation.

Brauer et al., (1986), found that Selected Ta is controlled centrally in a variety of ectotherms and endotherms with O_2 transport to the CNS the limiting factor. According to Brauer et al., (1986) hypoxia induces a decrease in selected Ta in both endotherms and ectotherms but the effect is not exerted upon thermogenesis as a common behaviour across a range of taxa. This lack of common effect was also evident in *M. domestica*. The physiological pathways involved in behavioural thermoregulation are unknown but are believed to potentially involve arginine vasopressin, histamine, lactate and adenosine (Wood and Malvin, 1992; Wood and Gonzales, 1996). Schurmann et al (1991) has also suggested that catecholamines may play a central role in the control of the selection of Ta during hypoxia. These mediators have not been studied in marsupials and so their role in behavioural thermoregulation can not be estimated. It would be of interest to measure such mediators in *M. domestica* to determine their role, if any, in behavioural thermoregulation.

8.3.3 Hypoxic Effects on Circadian Rhythms of Core Body Temperature and Selected Ambient Temperature

Normal circadian rhythms of core Tb are greatly affected by exposure to hypoxia in *M. domestica* with normal circadian patterns abolished by hypoxic ambient conditions. Circadian patterns of selected Ta are not apparent in normoxic conditions and the same holds true during hypoxia

with no circadian cycles evident in both species. However, patterns of Ta selection were different during hypoxia compared to normal conditions.

Hypoxia has been reported to affect thermoregulatory circadian patterns in humans (Vargas et al., 2001). In synchronised conditions of a 12:12-hour light-dark cycle, Mortola and Selfert (2000) also observed the elimination of circadian rhythms of Tb in adult rats, as hypoxia (10.5% O_2) blunted the Tb rise in the dark phase. Upon return to normoxia, Tb rapidly increased towards maximum normoxic levels and a normal circadian cycle of Tb regulation was then established. This pattern of returning to normal circadian cycles once re-exposed to normoxia was also evident in *M. domestica*. This indicates that both these marsupial species are able to return to a Tb within their normal circadian phase immediately after hypoxia exposure. Similarly, in free-running conditions (ie constant light) the Tb rhythm of adult rats was re-established at the expected phase of the cycle upon return to normoxia (Mortola and Selfert, 2000). This indicates that the action of hypoxia is at the hypothalamic centres of thermoregulation and not on the circadian clock. According to Gautier (1996), the hypothalamus is the key control centre of thermoregulatory responses to hypoxia. The results of this study certainly support this theory of hypothalamic control.

Following arousal from hibernation, hamsters tend to return to a normal preference for a warm environment however pre-hibernative hamsters show a marked preference of cool environments (Gumma and South, 1967). A similar response is also seen in rats which return to normal thermoregulatory behaviour patterns to Ta once hypoxia has been removed (Dupré and Owen, 1989). Immediately after hypoxia, *M. domestica* were returned to a normal 21% O_2 environment at which time they immediately returned to normal circadian rhythms of Tb and normal patterns of Ta selection. These observations of thermoregulatory behaviour indicate that recovery from hypoxia is immediate from a behavioural stance in mammals generally. This immediate change in behaviour may also greatly assist the prompt return of Tb to normal levels which is observed.

The effects of hypoxia on Tb and selected Ta in *M. domestica* were also analysed during two time periods within 24 hours. One period existed within the light phase (ie 1200-1800 hours)and the other within the dark phase (ie 0200-0800 hours) of the day. All animals displayed greater hypoxia-induced hypothermia during the light phase. This observation implies that during their most active periods (ie the dark phase) *M. domestica* does not actively utilise its environment to regulate Tb and, similarly, during the light phase (when relatively inactive) Ta selection by *M. domestica* is relatively unaffected by low ambient O_2 levels. This suggests that during hypoxia *M. domestica* use autonomic regulators to maintain hypothermic Tb.

The *M. domestica* utilised in this study were laboratory-bred. Consequently, these animals have had no experience of varying ambient conditions and so did not utilise the environment to benefit thermoregulatory strategies. Thus, little difference is observed between Selected Tas while normoxic and hypoxic in this species. This suggests that the use of the environment to modulate thermogenesis is a learned behaviour and does not indicate that hypoxia alters the set-point of thermoregulation. According to Brauer et al., (1986) there is evidence that temperature preference behaviour can be learned in the mouse and the lizard but not in fish, crustaceans and planaria. It is highly possible that thermoregulatory behaviour is a useful tool which marsupials can utilise as a strategy to maintain Tb when environmental conditions change. Such behaviour is probably learnt from previous experiences.

8.3.4 Effects of Hypoxia on Metabolic Rate in *M. domestica*

Hypoxia reduces metabolic rate reflecting the reduced activity of aerobic respiration. This reduction is marked in *M. domestica* as the Ta falls below the TNZ as seen in previous studies (eg Dupré et al., 1988; Gordon, 1993b; Gautier, 1996). Decreased thermogenesis during hypoxia results in hypometabolism with more profound effects seen in smaller animals due to their higher thermogenic requirements (Frappell et al., 1992; Saiki and

Mortola, 1996). Even invertebrates like molluscs exhibit metabolic suppression during hypoxia combined with a massive down-regulation of systemic O_2 delivery to match metabolic supply to demand (Boutilier et al., 2000). Hypoxia-induced metabolic rate is decreased in *M. domestica* to as low as 47% of the normoxic levels of metabolism; this is evident at a Ta of 35°C. At 5°C however metabolic rate is reduced by only 20% during hypoxic conditions. According to Gautier (1996) the magnitude of hypoxia-induced hypometabolism depends on Ta, the degree of hypoxia and the age and/or size of the animal. Ponies do not show a depressed metabolic rate in response to acute hypoxia (Korducki et al., 1994) and variable levels of metabolism have been observed in a number of mammalian species during hypoxia at room temperature (Frappell et al., 1992). As shown in Table 8.3.1 one of the smallest living marsupials (*S. crassicaudata*) depresses MR by 74% compared to a depression of 20% by a wallaby (*M. eugenii*) or 5% by a rat-kangaroo (*P. tridactylus*) (Frappell et al., 1992). *M. domestica* reduced metabolic rate by 31% at a Ta of 20°C (ie 0.83ml/g/hr to 0..57ml/g/hr) during hypoxia which compares favourably with the aforementioned results.

Table 8.3.1 Metabolic rates of eight marsupial species in normoxia and hypoxia at room temperature (data adapted from Frappell et al., 1992)

Species (common name)	n	Mass (g)	Ambient O_2 levels (%)	MR (ml/g/hr)
Sminthopsis crassicaudata	2	15	0.21	4.8
(fat-tailed dunnart)			0.10	1.2
Dasyuroides byrnei	1	107	0.21	2.5
(kowari)			0.10	1.1
Petaurus breviceps	2	147	0.21	1.8
(sugar glider)			0.10	0.9
Isoodon macrourus	2	945	0.21	0.9
(brown bandicoot)			0.10	0.5
Bettongia penicillata	2	1142	0.21	1.0
(brush-tailed bettong)			0.10	0.8
Potorous tridactylus	2	1262	0.21	0.7
(potoroo)			0.10	0.7
Macropus eugenii	2	3917	0.21	0.5
(tammar wallaby)			0.10	0.4

Advantages of inducing hypothermia and hypometabolism during hypoxia include improving O_2-loading in the lungs (due to a left shift of the oxyhaemoglobin dissociation curve), reducing energy-costing responses such as ventilation and resting tissue metabolism and brain cooling, thus preventing potential cell damage. *M. domestica* decrease both metabolism and Tb during hypoxia presumably to confer such advantages. Potential disadvantages of hypometabolism include ventricular fibrillation, interference with the normal immune response, the possible deceleration of brain development and the effects on metabolic rate during sympathetic activity (Wood and Malvin, 1992). However, the magnitude of hypometabolism will reflect the degree to which these disadvantages may affect a species. *M. domestica* reduced metabolism significantly but not dramatically during hypoxia therefore limiting any disadvantages induced by hypometabolism.

It can be concluded that *M. domestica* exhibit physiological hypothermia when exposed to hypoxia and that there is a threshold level of hypoxia (approximately 10% O_2 or above) below which selected Ta is significantly affected during short-term exposures. As reported in the literature, there are substantial intraspecies and interspecies variations in selected Ta during normoxia and hypoxia in laboratory experiments and this is also seen in marsupials. The results of this study suggest that generally, *M. domestica* regulates core Tb (and metabolism) at a lower level during long-term hypoxia by utilising a minimal amount of behavioural thermoregulation to prevent Tb decreasing too significantly. The *M. domestica* were laboratory-bred animals which may explain their minimal use of thermoregulatory behabiour. It is apparent that although all marsupials exhibit hypothermia in a hypoxic environment the mechanisms involved in this hypothermia may vary from species to species.

CHAPTER 9

THERMOREGULATORY RESPONSES TO SIMULTANEOUS EXPOSURE OF HYPOXIA AND BACTERIAL ENDOTOXIN

9.1 Introduction

It is well established that bacterial endotoxin generally increases core Tb (see Kluger, 1991) and hypoxia reduces core Tb (see Gautier, 1996) in many endotherms (including marsupials as shown in earlier chapters of this thesis). Furthermore, the effects of fever (ie a combination of increased heat production and decreased heat loss) involve the same underlying mechanisms as the effects of cold exposure while hypoxia reduces metabolic activity and therefore heat production. However the effects of hypoxia and fever in combination has not been addressed in many species. Such experiments may give some insight into the regulation of the thermoregulatory system and the significance of the hyperthermic and hypothermic responses seen during fever and hypoxia respectively. Thus, it was of interest to examine the effects of hypoxia on the observed fever response in *M. domestica*.

The underlying control of thermogenesis involves the hypothalamic set-point (Hammel et al., 1963). This set-point may be increased during a state of hyperthermia (Satinoff and Henderson, 1977) and decreased during a state of hypothermia (Gordon and Fogelson, 1991). If the general consensus is true and endotoxin increases the thermal set-point while chronic hypoxia decreases the thermal set-point then the induction of both simultaneously would provide some insight into the set-point theory.

From the previous experiments it appears that both LPS and hypoxia do not shift the set-point of thermoregulation in laboratory-bred *M. domestica*. If both bacterial endotoxin and hypoxia shift the set-point of

thermoregulation then their opposing effects will lead to a negligible change in thermoregulation dependant on the magnitude of the typical individual febrile and hypoxic responses. This is supported by Ricciuti and Fewell (1992) who found that the febrile response of young lambs to bacterial pyrogen was altered by hypoxemia with no change in core Tb observed. However, acute moderate hypoxia was found to depress the febrile response to endotoxin of guinea-pigs by reducing thermogenesis (Doherty and Blatteis, 1980). Similarly, the combined effect of LPS and hypoxia in rats resulted in a significant decrease in Tb (Almeida 1999). The mechanism of this ability of hypoxia to abolish febrile hyperthermic response remains unclear and clearly illustrates the need for further investigations. In addition, in mice, hypoxia has been shown to have an adverse effect on acquired endotoxin tolerance (Arya and Garcia, 1995). These authors further postulated that hypoxia may be a more potent stimulus than fever.

This study investigated the combined effects of hypoxia and LPS exposure on behavioural and autonomic thermoregulation in *M. domestica* and one *P. breviceps*. It was proposed that administering both hypoxia and endotoxin together would abolish the typically observed rhythm of body temperature and patterns of selected ambient temperatures usually produced by these conditions individually. A pronounced decrease in febrile hyperthermia was expected under hypoxic ambient conditions as it was proposed that hypoxia would suppress aspects of the febrigenic mechanism. Furthermore, since hypoxia and LPS each altered the thermoregulatory set-point in one *P. breviceps*, it was hypothesised that Ta preference in this animal (while febrile and hypoxic) would be similar to or slightly less than the range of Ta selected during control conditions.

9.2 Results

Appendix 8 illustrates continuous measurements of core Tb and selected Ta in each animal when injected with LPS and subsequently

exposed to hypoxia. Core Tb was affected by simultaneous LPS and hypoxia exposure with decreases in mean Tb observed in *M. domestica* and an increase in mean Tb observed in the *P. breviceps*. However, selected Ta was not affected by a combination of LPS and hypoxia in *M. domestica* despite a decrease in selected Ta observed in the *P. breviceps*. Tb and selected Ta recordings were compared to control (no LPS, normoxia), fever (LPS, normoxia) and hypoxia (no LPS, hypoxia) conditions during the same time period.

The Tb recordings for MOAG13 during LPS and hypoxia were affected by the transmitting signal from the implanted radiotransmitter in this animal which produced intermittent signals approximately 1.5 hours prior to the end of the experiment. At the end of the experiment the radiotransmitter in MOAG13 ceased transmitting any signal. Problems associated with Tb recordings during earlier fever experiments with MOAG17 meant that only a small time period (1.5 hours) could be used to compare Tb during LPS only and LPS+hypoxia in this animal.

9.2a Effects of LPS and Hypoxia on Core Body Temperature

A typical continuous recording of core Tb while exposed to LPS and hypoxia is illustrated in Figure 9.2.1 for *M. domestica* and Figure 9.2.2 for the *P. breviceps*. The mean core Tb of each animal was calculated while exposed to LPS and fever, and for the same time interval and time period of the day under control, fever and hypoxia conditions. These Tb recordings are shown in Table 9.2.1. All animals were exposed to a combination of LPS and hypoxia from 1215 hours to 1630 hours. During this time period, the core Tb of *M. domestica* was significantly lower than during control conditions ($t=22.1$; $p<0.05$) with a mean core Tb of 33.7±0.1°C (LPS and hypoxia) compared to a mean core Tb of 34.0±0.1°C during control conditions.

Figure 9.2.1 Typical Continuous Recordings of Body Temperature (Tb) from a *M. domestica* (MOAG18) - the effects of simultaneous exposure to LPS and hypoxia

[LPS was injected at 34.5 hours; hypoxia period was 36.5 hours to 42.6 hours]

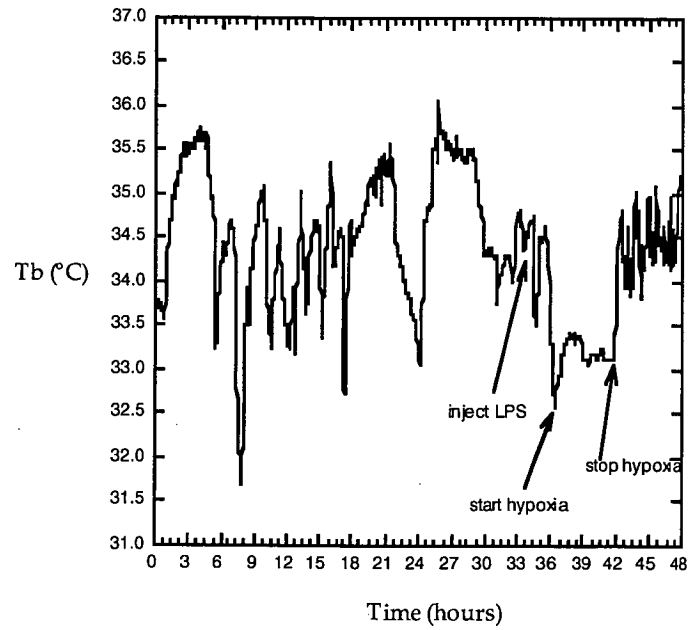


Figure 9.2.2 Continuous Recordings of Body Temperature (Tb) from a *P. breviceps* - the effects of simultaneous exposure to LPS and hypoxia

[LPS was injected at 33.5 hours; hypoxia period was 35.7 hours to 41.5 hours]

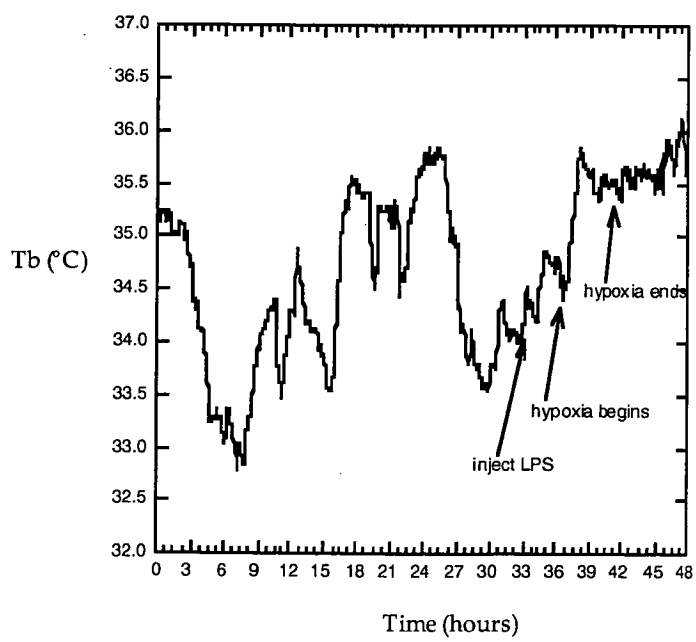


Table 9.2.1 Mean Core Body Temperature (Tb) and Mean Selected Ambient Temperature (Ta) of *M. domestica* and *P. breviceps* in response to control conditions, fever conditions, hypoxia conditions and simultaneous exposure to *E.coli* Lipopolysaccharide (LPS) and hypoxia (10-13% O_2)

Time period refers to the number of hours at which hypoxia exposure occurred which commenced 2 hours after the initial intramuscular injection of LPS.
* denotes significance difference from all other conditions.

Animal	Condition	Time Period (hours)	Core Tb (°C) (X±SD)	Selected Ta (°C) (X±SD)
MOAG13	control	5	35.2±0.2	28.2±5.6
	LPS only	5	35.0±0.3	34.7±1.3
	hypoxia only	5	34.2±0.4	29.5±1.9
	LPS+hypoxia	5	33.9±0.6*	27.7±4.2*
MOAG15	control	5	33.8±0.4	28.5±1.6
	LPS only	5	34.6±0.4	29.0±1.5
	hypoxia only	5	32.7±0.4	27.9±1.2
	LPS+hypoxia	5	33.4±0.2*	28.6±1.5*
MOAG16	control	6	34.3±0.3	29.3±2.1
	LPS only	6	34.8±0.4	28.4±1.7
	hypoxia only	6	34.2±0.2	27.8±1.6
	LPS+hypoxia	6	34.1±0.3*	31.7±1.7*
MOAG17	control	6	34.3±0.2	32.3±2.5
	LPS only	2	34.6±0.2	33.2±1.3
	hypoxia only	6	34.2±0.2	31.9±2.0
	LPS+hypoxia	2	34.0±0.1	29.5±5.2
	LPS+hypoxia	6	34.2±0.2	32.3±2.0*
MOAG18	control	6	34.2±0.5	30.3±3.7
	LPS only	6	34.5±0.3	27.8±2.4
	hypoxia only	6	33.5±0.3	26.6±1.6
	LPS+hypoxia	6	33.2±0.3*	28.9±2.0*
PETAG1	control	5.75	34.4±0.5	33.8±1.3
	LPS only	5.75	34.7±0.4	20.4±1.9
	hypoxia only	5.75	33.6±0.7	21.8±6.0
	LPS+hypoxia	5.75	35.3±0.4*	31.4±1.7*

In comparison to hypoxia conditions during the same time period, core Tb was also significantly lower for *M. domestica* ($t=6.5$; $p<0.05$) although the decrease was not as significant and in one animal (MOAG17), no significant difference was noted ($t=1.8$; $p>0.05$). During fever conditions, a significantly higher Tb was recorded ($t=40.2$; $p<0.05$ for time period 1215-1345; $n=5$ and $t=-41.9$; $p<0.05$ for time period 1215-1630; $n=4$) compared to that recorded during a combination of LPS and hypoxia in *M. domestica.*

A significant increase in core Tb was noted in PETAG1 during LPS and hypoxia with a change from 34.4±0.5°C (control conditions) to 35.3±0.4°C ($t=-25.7$; $p<0.05$). Core Tb during LPS and hypoxia was also significantly higher than Tb recordings during fever conditions ($t=-17.6$; $p<0.05$) and hypoxia conditions ($t=-39.8$; $p<0.05$) in PETAG1.

The effect of combined LPS and hypoxia on the circadian rhythms of core Tb in each animal was analysed using Fourier analysis. Tb recordings at one minute intervals for each animal were analysed using a single series Fourier analysis and results plotted by period using transformations in which the mean was subtracted and detrended and period values corresponding to the largest peaks were determined. Results of this analysis for each animal are presented in Appendix 13. As the animals were not exposed to a combination of LPS and hypoxia for 24 hours, the circadian rhythm was only affected at a certain time period of the day (ie during the exposure to LPS and hypoxia). The normal pattern of Tb seen in control conditions was disrupted by a combination of LPS and hypoxia in all animals although most animals were able to maintain a 24 hour period. This is illustrated in Table 9.2.2 which shows the effect of a combination of LPS and hypoxia on the amplitude, acrophase, and period of Tb in each *M. domestica* and PETAG1. The 24-hour rhythms maintained by MOAG15, MOAG17, MOAG18 and PETAG1 were not as defined as those seen under control conditions (see Appendix 13). Individual animals also exhibited additional rhythms, for example, MOAG18 showed an additional rhythm of Tb at 6, 8 and 16 hours as illustrated in Appendix 13.

Table 9.2.2 The effects of a Combination of LPS (Lipopolysaccharide) and Hypoxia on Core Body Temperature: Amplitude, acrophase (timing of peak), and period
[Period was determined by Fourier analysis; amplitude is the difference between the maximum and minimum Tb during the exposure; acrophase is given in 24-hour time]

Animal	Condition	Min. (°C)	Max. (°C)	Amplitude (°C)	Acrophase	Period (hours)
MOAG13	Control	33.0	35.0	2.0	13:52	
	LPS+Hypoxia	32.6	35.1	2.5	17:22	9.6
MOAG15	Control	33.1	34.2	1.1	14:57	
	LPS+Hypoxia	32.9	33.9	1.0	11:41	24
MOAG16	Control	33.3	34.4	1.1	12:14	
	LPS+Hypoxia	33.7	34.6	0.9	11:20; 11:23; 14:17; 14:20; 14:23; 14:26; 14:35	48
MOAG17	Control	33.2	34.6	1.4	17:34	
	LPS+Hypoxia	33.8	34.6	0.8	15:31	24
MOAG18	Control	34.3	34.7	0.4	11:35	
	LPS+Hypoxia	32.6	34.4	1.8	11:43	24
PETAG1	Control	33.8	34.9	1.1	13:55	
	LPS+Hypoxia	34.4	35.8	1.4	14:06	24

9.2b Effects of LPS and Hypoxia on Selected Ambient Temperature

A typical continuous recording of selected Ta while exposed to LPS and hypoxia is illustrated in Figure 9.2.3 for *M. domestica* and Figure 9.2.4 for PETAG1. The mean selected Ta of each animal was determined during the time period of simultaneous LPS and hypoxia exposure and similarly under control conditions, hypoxia conditions and fever conditions as shown in

Table 9.2.2. Selected Ta was also analysed during a common time period (1215 to 1630 hours) to determine differences between different animals. There was significant difference between selected Ta during control conditions and LPS+hypoxia in *M. domestica* ($t=-10.0$; $p<0.05$). An even more significant decrease in selected Ta was observed for the *P. breviceps* during simultaneous exposure to LPS and hypoxia ($t=65.8$; $p<0.05$). *M. domestica* selected a mean selected Ta of 29.9±0.9°C during LPS+hypoxia (compared to a mean selected Ta of 29.6±1.3°C in control conditions). This was lower but comparable to a selection of 31.4±1.7°C while exposed to concurrent LPS and hypoxia for PETAG1.

Compared to hypoxia during the same time period, different patterns of Ta selection were observed with *M. domestica* selecting significantly lower Tas ($t=-41.9$; $p<0.05$) and the *P. breviceps* selecting significantly higher Tas ($t=-91.0$; $p<0.05$) during LPS+hypoxia. Similarly, *M. domestica* selected significantly higher Tas during fever conditions ($t=40.2$; $p<0.05$ for time period 1215-1345 hours; $n=5$ and $t=20.6$; $p<0.05$ for time period 1215-1630 hours; $n=4$) and the *P. breviceps* selected significantly lower Tas during fever conditions ($t=-261.0$; $p<0.05$) compared to LPS+hypoxia.

Figure 9.2.3 Typical Continuous Recordings of Selected Ambient Temperature (Ta) from a *M. domestica* (MOAG18) - the effects of simultaneous exposure to LPS and hypoxia

[*LPS was injected at 10.6 hours; hypoxia period was 12.5 hours to 18.6 hours]

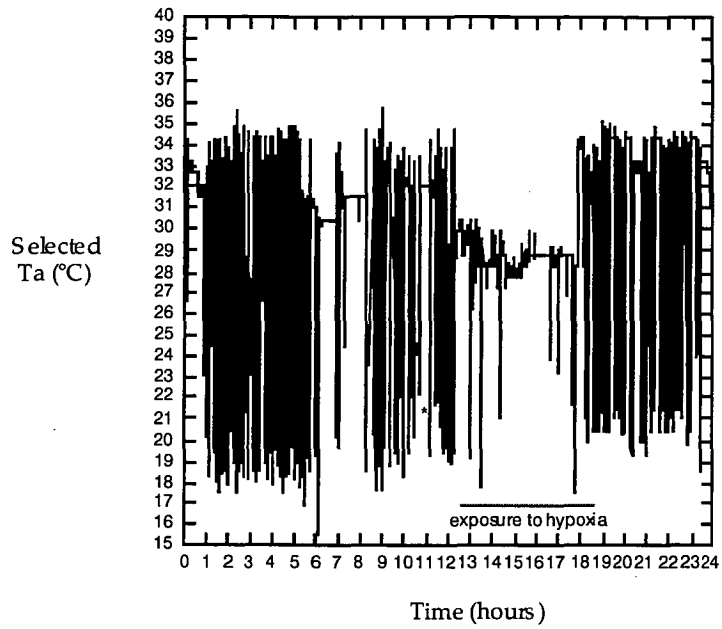
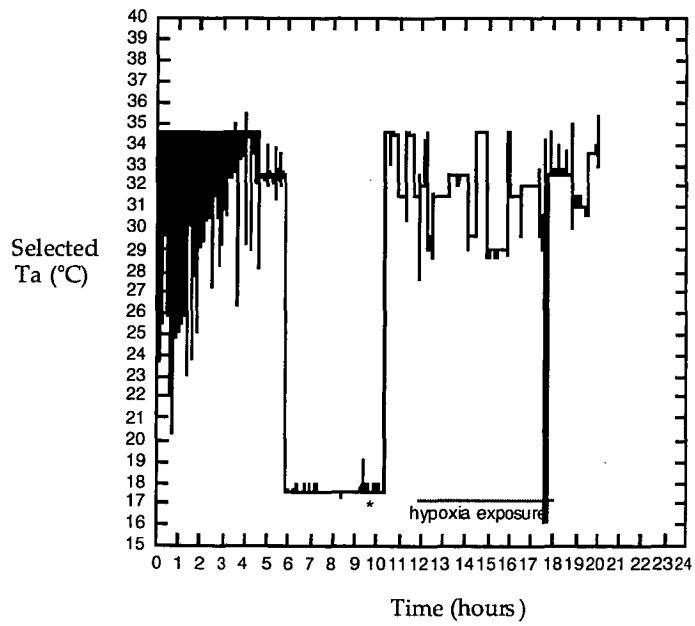


Figure 9.2.4 Continuous Recordings of Selected Ambient Temperature (Ta) from a *P. breviceps* - the effects of simultaneous exposure to LPS and hypoxia

[*LPS was injected at 9.5 hours; hypoxia period was 11.8 hours to 17.5 hours]



The effect of combined LPS and hypoxia on the circadian rhythm of selected Ta in each animal was analysed using Fourier analysis (StatSoft STATISTICA 4.1). Selected Tas recorded every 6 seconds were analysed using a single series Fourier analysis as for Tb analysis to determine the effects of fever and hypoxia on patterns of selected Ta. Results of this analysis for each animal are presented in Appendix 13. Similarly to fever conditions and hypoxia conditions, a combination of LPS and hypoxia disrupted patterns of temperature selection. Tas selected at periods of the day when the animals were exposed to LPS and hypoxia were different to those selected under control conditions as already mentioned.

The frequency of responses to each Ta available in the thermal gradient was also determined during control conditions and during LPS+hypoxia exposure for each *M. domestica* as shown in Figures 9.2.5 (a-e). Typically, the selection of Ta by individual animals was variable, with *M. domestica* tending to select a similar range of Ta during control (normoxic) conditions and LPS+hypoxia. Typically, during LPS+hypoxia, *M. domestica* favoured Tas around 28°C, 29°C and 33°C and the *P. breviceps* favoured Tas around 29-35°C. Figure 9.2.6 shows the mean frequency of responses to Ta under control and LPS+hypoxia for *M. domestica*.

PETAG1 however spent considerably more time at higher Tas during fever+hypoxia as illustrated in Figure 9.2.7. The frequency of responses to Ta during LPS and hypoxia compared to during fever and during hypoxia are shown in Figures 9.2.8a for *M. domestica* and 9.2.8b for the *P. breviceps*. The combination of LPS and hypoxia caused individual animals to select warmer Tas with cooler Tas selected during hypoxia only (ie with no combination of LPS).

Figure 9.2.5a The effect of fever and hypoxia on the frequency of responses to ambient temperature in MOAG13

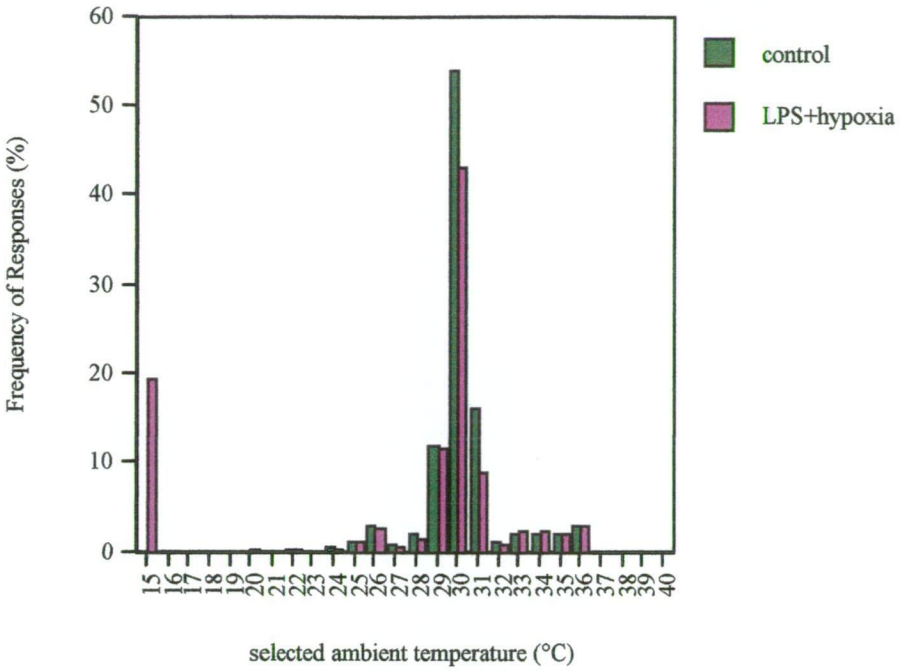


Figure 9.2.5b The effect of fever and hypoxia on the frequency of responses to ambient temperature in MOAG15

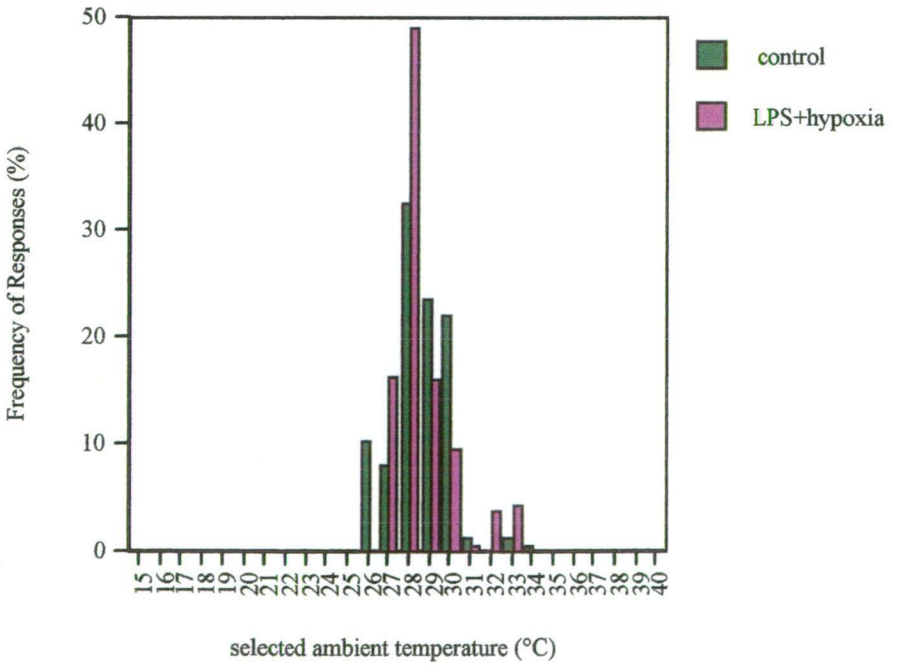


Figure 9.2.5c The effect of fever and hypoxia on the frequency of responses to ambient temperature in MOAG16

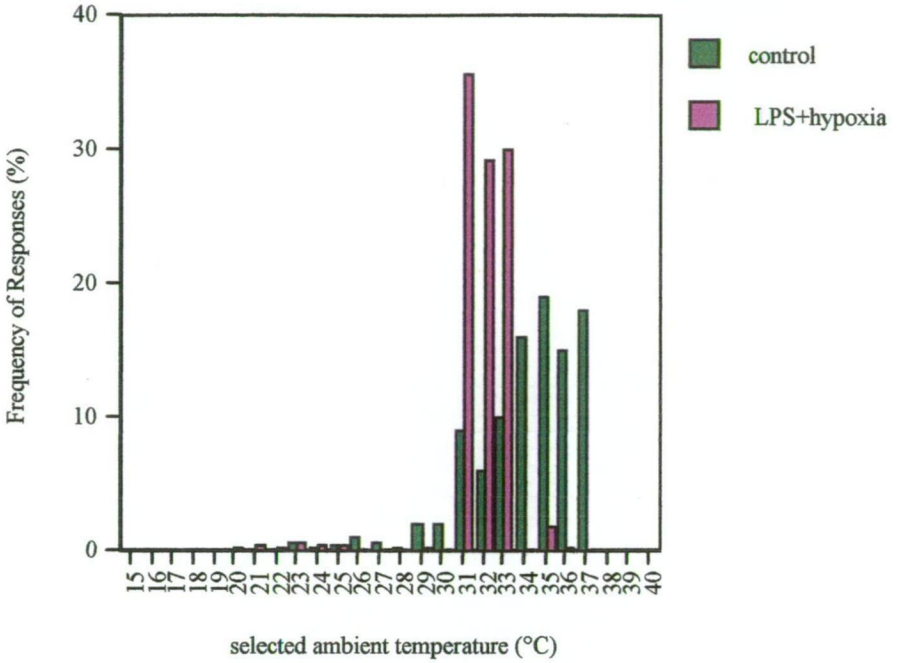


Figure 9.2.5d The effect of fever and hypoxia on the frequency of responses to ambient temperature in MOAG17

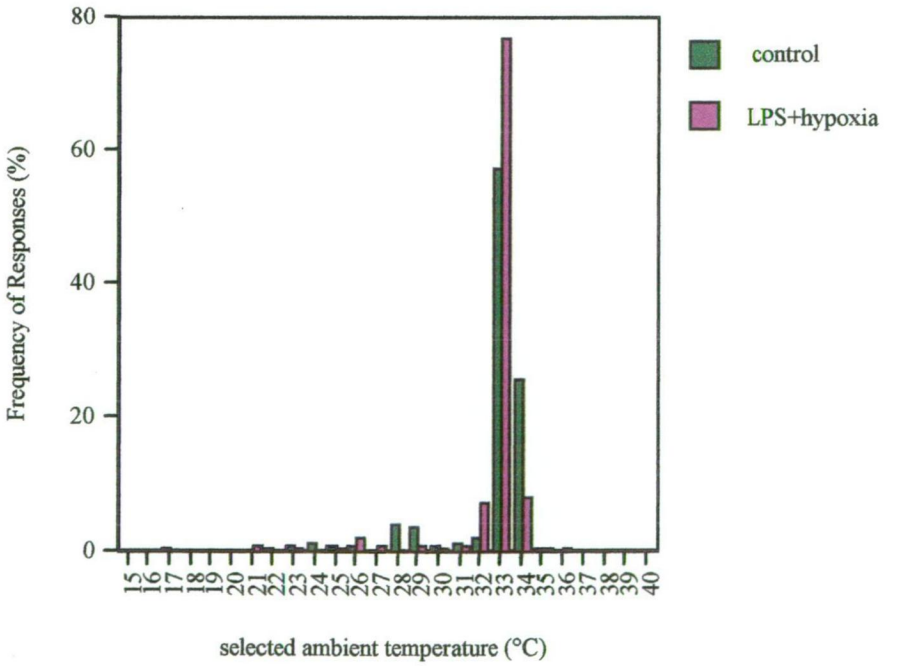


Figure 9.2.5e The effect of fever and hypoxia on the frequency of responses to ambient temperature in MOAG18

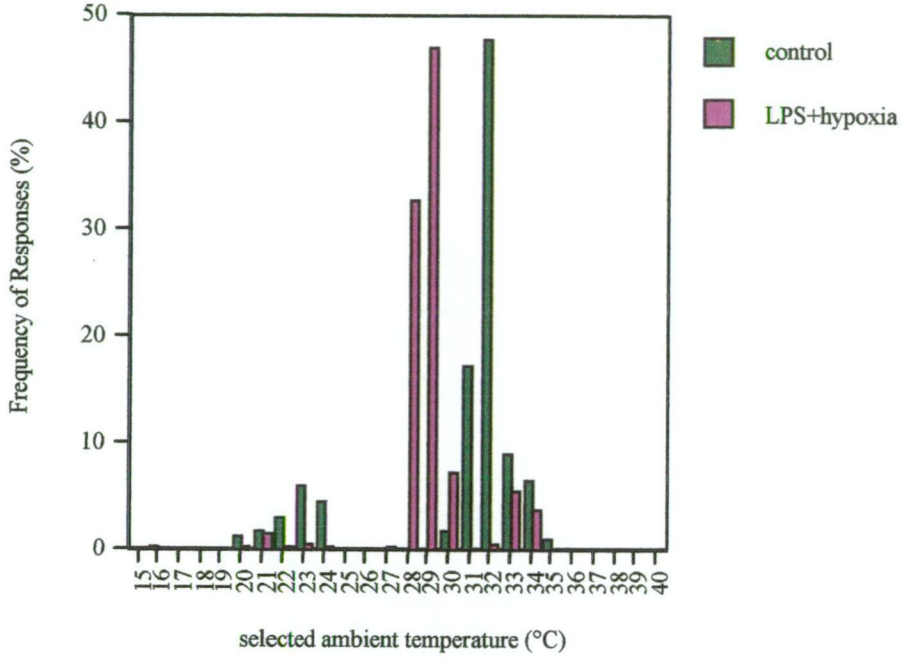


Figure 9.2.6 Mean frequency of responses to ambient temperature after exposure to lipopolysaccharide (LPS) and while exposed to hypoxia in *M. domestica*

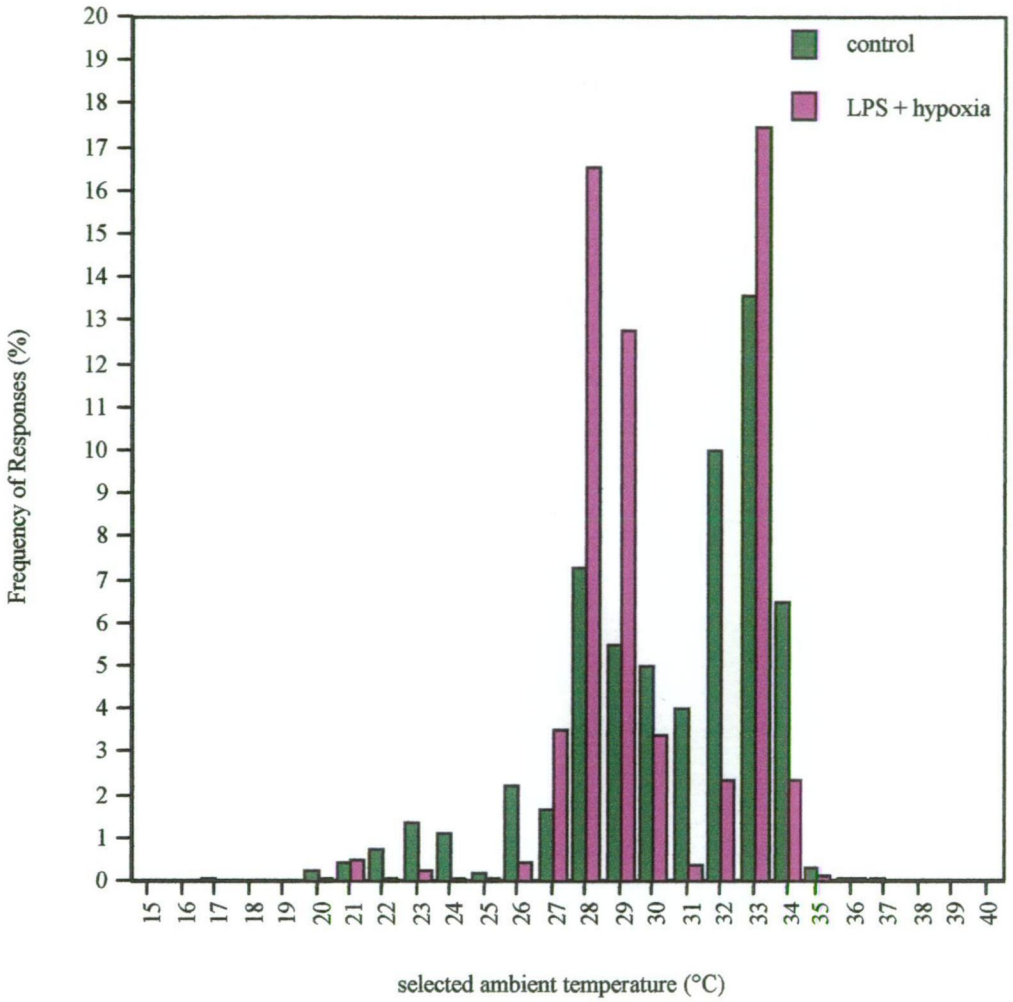


Figure 9.2.7 Frequency of responses to ambient temperature after exposure to lipopolysaccharide (LPS) and while exposed to hypoxia in PETAG1

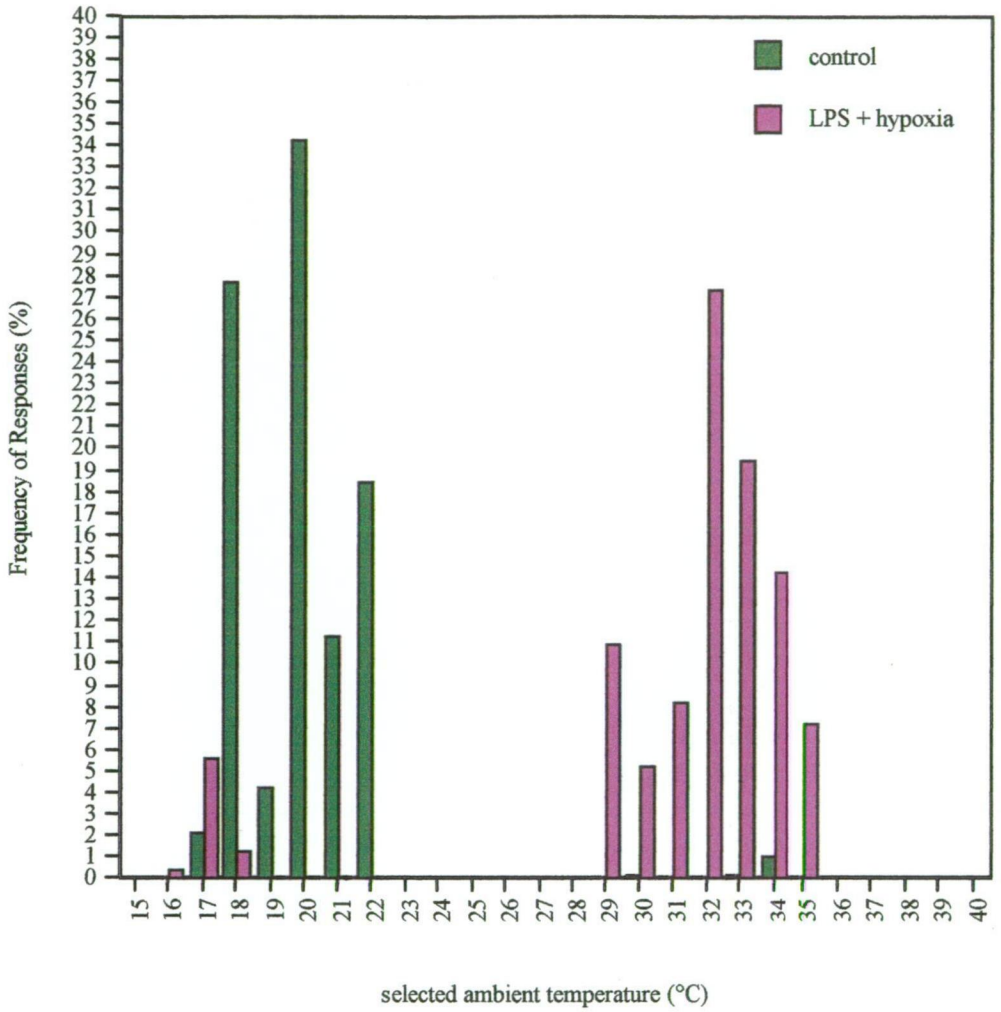


Figure 9.2.8a Comparative effects of fever (LPS), hypoxia and a combination of fever and hypoxia on the frequency of responses to ambient temperature in *M. domestica* (n=5)

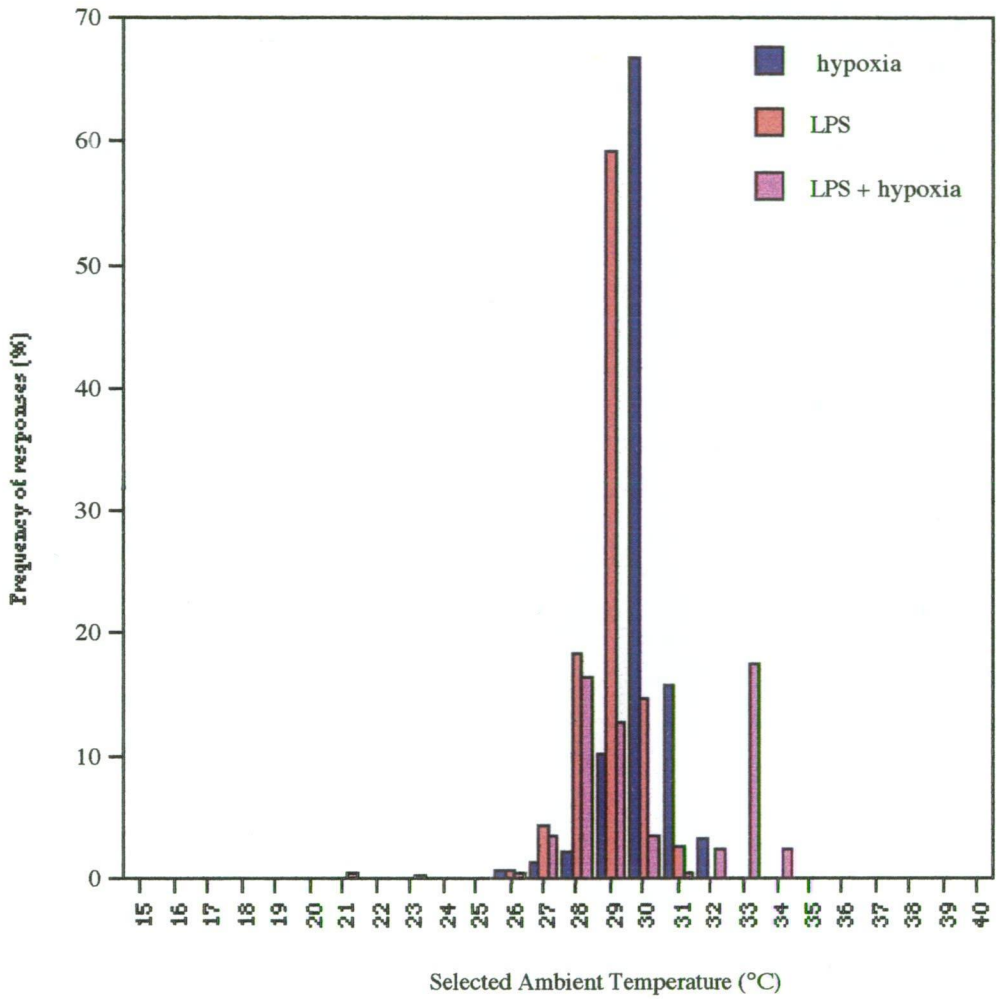
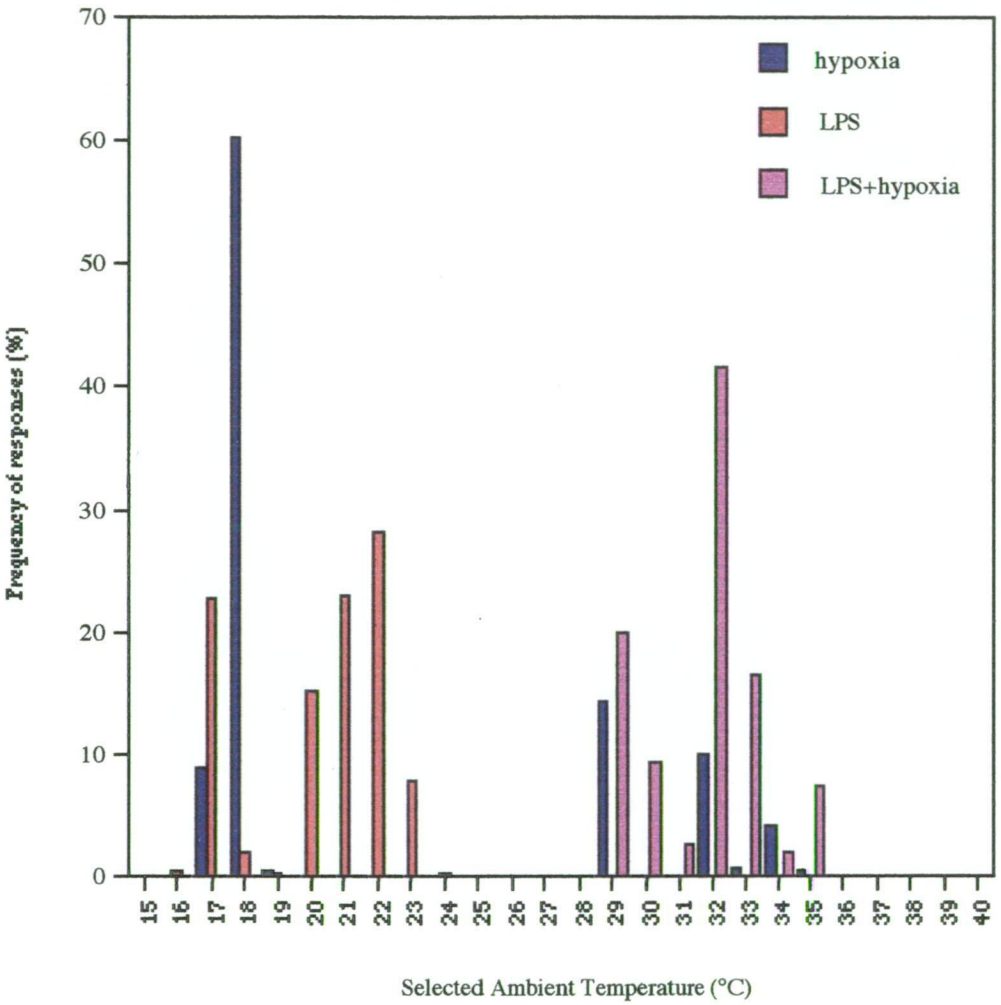


Figure 9.2.8b Comparative effects of fever (LPS), hypoxia and a combination of fever and hypoxia on the frequency of responses to ambient temperature in one *P. breviceps*



9.3 Discussion

To my knowledge, only six investigations have previously been published on the combined effects of fever and hypoxia in mammals. In the majority of these investigations, hypoxia markedly reduced the febrile response particularly with respect to the regulated increase in Tb characteristic of mammalian fever (Farkas et al., 1966; Doherty and Blatteis, 1980; Riciutti and Fewell, 1992; Almeida et al., 1999). According to Wood and Malvin (1992) if there is a limited supply of oxygen or a limitation in oxygen-carrying capacity then the fever of hyperthermia will aggravate hypoxemia. According to the results obtained in this study, core Tb and selected Ta are altered under the influence of an endotoxin fever in combination with hypoxia. The expected increase in core Tb induced by bacterial endotoxin is reduced by hypoxic exposure in *M. domestica* which is in agreement with previous observations in rabbits (Cheymol and Levassort, 1955), rats (Farkas et al., 1966; Almeida et al., 1999), guinea pigs (Doherty and Blatteis, 1980) and lambs (Ricciuti and Fewell, 1992). In addition, Arya and Garcia (1995) have suggested that hypoxia/reoxygenation diminishes LPS-induced tolerance of macrophages in mice.

The benefits of preventing the hyperthermic response of fever while subject to a hypoxic environment may be interpreted as a survival mechanism. It is well documented that the typical hypothermic response seen during hypoxia results in reductions in ventilation, resting tissue metabolism, and improvements in oxygen loading in the lungs. A simultaneous hyperthermic response during fever obviously eliminates these advantages. LPS-induced fever involves an increase in heat production through the possible stimulation of endogenous pyrogens such as PG. These endogenous pyrogens stimulate heat production by a variety of mechanisms including the activation of sympathetic activity to BAT thus promoting NST. As hypoxia depresses the febrile response in many

endotherms it is apparent that a low ambient oxygen level serves to decrease heat production by inhibiting endogenous pyrogen activity.

Suppression of fever by low ambient O_2 levels was first demonstrated in rabbits at 15-25°C by Cheymol and Levassort (1955). Similarly, Farkas et al., (1966) demonstrated that a 12% O_2 environment prevented a rise in Tb and VO_2 in rats at 20°C and at 30°C the rise in Tb was prevented but VO_2 increased. Doherty and Blatteis (1980) observed significant increases in core Tb in adult guinea pigs at 25°C following administration of bacterial pyrogen (*S. enteritidis*) during hypoxemia (although to a lesser extent to that observed in room air) and found that exposure to hypoxia significantly decreased typical increases in VO_2. Furthermore, the reduction in VO_2 during hypoxia accounted for the reduced fever as changes in VO_2 preceded corresponding changes in Tb in both room air and hypoxia (Doherty and Blatteis, 1980). *M. domestica* similarly had a reduced febrile response during hypoxia. However, hypoxia failed to blunt the hyperthermic response to LPS in the *P. breviceps* but rather exaggerated it. These observations indicate that VO_2 would also be reduced in *M. domestica* during simultaneous exposure to LPS and hypoxia as previously observed in guinea pigs by Doherty and Blatteis (1980).

Ricciuti and Fewell (1992) did not find significant increases in core Tb in response to bacterial pyrogen in young lambs when exposed to hypoxia. The explanation for this latter finding was suggested to be related to the dependance of lambs on NST to develop a fever as NST is selectively inhibited during hypoxic exposure (eg Blatteis and Lutherer, 1973). According to Doherty and Blatteis (1980), guinea pig fever also involves a small but significant NST component and the intensity of shivering during hypoxia is more vigorous than in room air. In lambs, shivering and vasoconstriction are sustained during fever and hypoxia (Ricciuti and Fewell, 1992). This is despite a lack of hyperthermia in these animals typical of fever and a lack of increase in total body VO_2 during hypoxia. It is thus apparent that shivering and vasocontriction alone are insufficient to raise

core Tb in lambs following pyrogen administration. NST must therefore play a key role in the elevation of core Tb. This elevation is limited by hypoxia as it limits O_2 delivery to BAT and prevents NST required for fever production.

The presence of BAT in marsupials is still controversial although it seems likely that noradrenaline-mediated NST through skeletal muscle in non-macropod marsupials does exist (eg Nicol et al., 1997; Rose et al., 1999). Shivering was not visually observed during the current experiments and other mechanisms of heat production were not assessed. As the *P. breviceps* increased Tb significantly during a combination of LPS and hypoxia exposure in comparison to previous fever responses it can be deduced that hypoxia probably had no inhibitory effect in this animal (or alternatively any inhibitory effect is counteracted by thermal behaviour). This result was unusual as inhibition of fever by hypoxia was observed in all *M. domestica.* This latter observation indicates that NST may be an underlying mechanism of marsupial fever which may be inhibited during hypoxia. This NST would occur in skeletal muscle in *M. domestica* as BAT has not been identified in thie species (Hayward and Lisson, 1992).

The action of hypoxia on pyrogenesis in both *M. domestica* and the *P. breviceps* was direct when considering the immediate change in Tb patterns once the air in the gradient was switched from normoxia to hypoxia. This was similarly observed by Doherty and Blatteis (1980). The immediate change in Tb was also accompanied in many animals by an immediate change in Ta preference. This may be due to agitation of the animals by the reduction in ambient oxygen levels or a thermoregulatory adaptation. Doherty and Blatteis (1980) recorded VO_2 as well as rectal temperatures and deduced that the reduced VO_2 observed during hypoxia accounted for the lowered fever. The reduced hyperthermic response in *M. domestica* may also be due to a decrease in VO_2.

Hyperventilation may be, in part, responsible for the attenuated fever seen during hypoxia in lambs (Ricciuti and Fewell, 1992). During hypoxia, minute ventilation is usually increased although hypometabolism is more prominent in mammals (Frappell et al., 1992). This would increase heat dissipation via the respiratory tract which may overwhelm any attempt to increase Tb in response to bacterial endotoxin. It is possible that an increased ventilation rate was responsible, at least in part, for the blunted febrile response seen in *M. domestica*.

Nitric oxide pathways in the central nervous system of mammals have also been shown to be involved in responses to LPS and hypoxia. Nitric oxide is required for the production of fever (Scammell et al., 1996; Soszynski, 2001; Steiner and Branco, 2001) and central nitric oxide causes a reduction in Tb during hypoxia (Branco et al., 1997). In addition, in rats, the inhibition of fever by hypoxia has been proposed to be related to nitric oxide (Almeida et al., 1999; Nakano et al., 2001). Nitric oxide pathways have not been investigated in marsupials however it is reasonable to postulate that such a pathway may also be an underlying mechanism of thermogenic adjustments to LPS and hypoxia. As marsupials exhibit comparable changes in thermogenesis to mammals when exposed to LPS and/or hypoxia, it is proposed that the underlying mechanisms will also be similar. An investigation of the role of nitric oxide pathways in marsupial thermoregulation would confirm these assumptions.

Febrile responses in mammals have been reported to be altered not only by changes in ambient O_2 levels but also by helox environments and high CO_2 environments. Vaughn et al., (1980) demonstrated a dose-dependant alteration in the febrile response of rabbits when exposed to helox. The dose-dependant differences were proposed by these authors to be due to an interaction between the helox-induced increase in heat loss and an increase in "fever drive". The hyperthermic response of fever has been found to be delayed by hypercapnia (as a result of lower heat production) in the cat (Sachdeva and Jennings, 1994). This hypercapnic effect is quite

different to the typical hypoxic effect observed in mammals (including marsupials) where the typical hyperthermic response of fever is abolished rather than simply delayed. Such studies indicate that environmental conditions (such as variations in pO_2 and pCO_2) affect the magnitude of fever in mammals.

Patterns of Ta selection were recorded for both *M. domestica* and the *P. breviceps* during a combination of LPS and hypoxia. There are no previous investigations of the effects of fever and hypoxia simultaneously on behavioural thermoregulation in any other mammal and it was proposed that a negligible effect would be seen, particularly in the *P. breviceps*. However, the combination of LPS and hypoxia exposure resulted in a significant increase in the selected Ta of the *P. breviceps* compared to selected Ta during fever or hypoxia only and a significant decrease in selected Ta compared to control conditions. The combination of hypoxia and LPS had a less apparent effect on Ta preference in *M. domestica* with a slight but significant decrease in mean selected Ta during these conditions compared to the mean selected Ta in control conditions, fever conditions or hypoxia conditions. However, *M. domestica* did spend a considerable amount of time at higher Tas during LPS and hypoxia as shown in Figure 9.2.12. A preference for higher Ta in the *P. breviceps* was noticeable approximately 25 minutes after LPS was injected into the animal and was maintained when ambient O_2 levels were reduced from 21% to 10-12%. It is possible that this selection of higher Ta in the *P. breviceps* contributed considerably to the increase in core Tb also observed in this animal. Utilising behaviour rather than relying solely on physiological adjustments to alter Tb can be interpreted as an energy-saving mechanism in this animal.

Behavioural thermoregulation has traditionally been shown to vary among different mammalian species although temperature-seeking behaviour is primarily utilised by ectotherms to maintain Tb. In addition to autonomic regulators, however, behaviour is a useful tool to adjust core Tb when environmental conditions (other than temperature) change. Selection

of cooler temperatures indicates a reduction in the set-point of thermoregulation. Similarly, the selection of warmer temperatures indicates an increase in the thermoregulatory set-point. If hypoxia inhibits fever (as seen in Tb responses in rats and in *M. domestica*) then the selection of cooler Tas with simultaneous decreases in core Tb would suggest a resetting of the set-point of thermoregulation in the hypothalamus.

Generally *M. domestica* selected higher Tas during fever and hypoxia than during control, hypoxia or fever conditions. This indicates that there is no resetting of the hypothalamic set-point under these conditions. This may particularly be due to the fever component of the thermoregulatory response. During hypoxia alone Tb is decreased and therefore a resetting of the hypothalamic set-point would result in a preference for lower Ta as seen in *M. domestica*. LPS increases Tb and so an increase in selected Ta would be expected. The febrile response induced by LPS in *M. domestica* (ie increased core Tb) is attenuated by hypoxia yet the behavioural selection of Ta in this species is not similarly affected. The preference to remain in higher temperatures while febrile and in a hypoxic environment suggests that this species is utilising the environment to retain a higher Tb typical of fever while exposed to hypoxia which will induce hypothermia.

The *P. breviceps* used in this study was an animal captured in its natural environment and therefore capable of utilising the environment to maintain its Tb moreso than the laboratory-bred *M. domestica*. The use of thermal behaviour enabled the *P. breviceps* to increase Tb (due to LPS) and overcome any hypoxia-induced hypothermia yet *M. domestica* were not able to utilise the environment as effectively to similarly overcome these hypothermic effects. This may be because *M. domestica* had been bred and maintained in a constant condition environment (ie 28-30°C) and were therefore unable to utilise Ta to alter Tb.

Alternatively, behaviour has little effect on thermoregulation in *M. domestica* and the different core Tb responses observed in *M. domestica* and

the *P. breviceps* are purely due to differences in autonomic regulation of Tb. This may also be possible as behavioural selection of Ta, although higher than that selected during LPS or hypoxia only, was actually lower than that observed under control conditions. The true role of thermoregulatory behaviour is still far from understood in marsupial thermoregulation and needs to be addressed in mammalian studies of thermoregulatory effects during LPS and hypoxia. In these studies, where possible, behavioural thermoregulation should be investigated using animals that have not been laboratory-bred.

In summary, *M. domestica* significantly suppresses hyperthermic responses to LPS when exposed to hypoxia. This was not seen in the *P.breviceps*. In addition, environmental temperature may play a role in Tb maintenance in response to a combination of LPS and hypoxia in marsupials. The ability of hypoxia to attenuate the febrile response to LPS is apparent in *M. domestica* and indicates the strength of hypoxia as a stimulus to thermogenic mechanisms in this species. As a typical hyperthermic febrile response was not observed it can be suggested that this species may contain a NST component of thermoregulation which is selectively inhibited during hypoxia. Like lambs, and to a lesser degree, guinea pigs, marsupials may depend on NST to develop fever (although this was not apparent in the *P. breviceps*). It is also possible that marsupials, and indeed other mammals, may utilise environmental temperature to adjust changes in core Tb.

These experiments have provided an insight into the thermogenic effects of hypoxia during LPS fever in *M. domestica* (although somewhat inconclusively). It is proposed that this species copes with O_2 supply and demand during fever by tending to utilise autonomic mechanisms to respond to the hypoxic conditions and behaviour to adapt to the LPS. This enables them to effectively maintain aerobic metabolism and normal thermoregulation despite the presence of a mediator of hyperthermia (ie LPS) and the hypothermic effect of the environment (ie hypoxia). The underlying mechanisms of these effects however remain to be determined.

CHAPTER 10

GENERAL DISCUSSION

This study has investigated the concept of a thermoregulatory set-point in marsupials by determining circadian rhythms of core Tb in adult and juvenile animals and the effect of Ta, endotoxin and hypoxia on these rhythms. The study has mainly focussed on a laboratory-bred species, *Monodelphis domestica*. Some data from one *Petaurus breviceps* (obtained from its natural habitat) has also been included for comparative purposes.

An ability to maintain a constant Tb despite fluctuations in environmental conditions is a feature common to endotherms including mammals such as marsupials. Numerous studies have indicated that insulation, shivering and non-shivering mechanisms, heat production through locomotor activity, physiological controls (ie vasomotor) and behaviour all play an integrative role in endothermic thermoregulation. Marsupials are endothermic and although they may lack certain thermoregulatory mechanisms such as BAT-induced NST (small amounts of BAT may occur in some small species), they are efficient thermoregulators utilising both behavioural and autonomic mechanisms.

This study has aimed to assess the thermoregulation of a small non-macropod marsupial (*M. domestica*) including normal circadian Tb rhythms, the role of thermoregulatory behaviour and ability of the species to thermogenically adapt to injections of bacterial LPS, reduced ambient O_2 levels (hypoxia) and exposure to both LPS and hypoxia simultaneously. In addition, the effects of cold Tas on warm-acclimated, laboratory-bred *M. domestica* were investigated and thyroid hormone levels measured. A circadian rhythm of Tb typical of a nocturnal mammal is exhibited in *M. domestica* as previously reported in other marsupials such as *T. vulpecula* (Gemmell and Cepon, 1993). The investigation has also successfully demonstrated a typical mammalian febrile response in *M. domestica* and

highlighted some thermoregulatory mechanisms used by this marsupial species when exposed to various ambient conditions.

M. domestica is a nocturnal marsupial and has a circadian rhythm of Tb typical of most mammals (both eutherian and marsupial). This rhythm is highlighted by peaks of core Tb during the nocturnal phase and decreases in core Tb during the diurnal phase. The basis of this circadian pattern is assumed to be hypothalamic although, according to Refinetti (1998), the homeostatic control of Tb is exerted independently from the circadian control. When initially exposed to colder than normal Tas this rhythm of Tb in *M. domestica* is affected indicating a disruption to hypothalamic control and the thermoregulatory set-point by the cold. Previous studies have suggested that the autonomic response of cold-induced thermogenesis opposes rather than defends the normal circadian rhythms of Tb (eg Refinetti, 1998). This may also be true in marsupials although *M. domestica* does demonstrate an ability to acclimatise to colder Tas as this species does maintain rhythms of Tb during subsequent exposures to cold Tas. This indicates that the initial exposure to cold conditions sensitises the hypothalamic control of Tb. This may involve mediators of the central nervous system such as PG as such mediators are known to play a role in the production of fever in the mammalian brain (eg Satinoff et al., 1999) and may also be involved in normal Tb control (Fraifeld et al., 2001).

Behavioural thermoregulation (ie the selection of selected Tas) is utilised by *M. domestica* however it has no set circadian pattern despite its apparent influence on Tb. This indicates that although marsupials are not fully reliant on Ta as a form of thermoregulation, as seen in ectothermic species (eg Peterson, 1987), they do nevertheless display an evolutionary remnant of this form of thermoregulation and seek selected Tas during intermittent phases of core Tb control. The lack of a common Ta rhythm in *M. domestica* could be interpreted to suggest that the rhythm of Ta selection has no physiological function however evidence from this study suggests otherwise. In addition, when allowed to preferentially seek Ta *M. domestica*

varies Tb over a lower range than when exposed to a constant Ta. Despite this, mean Tb is maintained irrespective of temperatures available in the immediate environment. This indicates that thermoregulatory control is based primarily on autonomic mechanisms in marsupials however behaviour may be utilised to reduce the energy costs of thermogenesis.

The full use of thermoregulatory behaviour in marsupials needs further investigation with particular focus on free-living species. In addition, it is known that food deprivation modulates thermoregulation in mammals and birds (eg Yoda et al., 2000) yet this has not been investigated in marsupials. Significant phase shifts in the endogenous Tb rhythm of starved Japanese quails have been observed (Underwood et al., 1999) yet how food deprivation affects the daily cycle of mammalian thermoregulation is largely unknown (Yoda et al., 2000). Investigations incorporating Selected Ta in a thermal gradient and food deprivation have not been investigated but would be of great interest in understanding thermoregulatory behaviour in mammalian species including marsupials.

This study has successfully demonstrated that *M. domestica* exhibit a fever response to bacterial LPS which is commonly observed in many higher vertebrates. Tb is significantly increased by intramuscular injections of *E. coli* LPS and Tb rhythms are consequently disrupted in *M. domestica* during the time period that LPS-induced hyperthermia occurs. In *M. domestica*, the duration of fever was quite short therefore making it difficult to determine the full impact of the fever on the normal rhythm of Tb. The short fevers seen in this study did not eliminate the 24-hour rhythm of Tb, however disruptions to the daily patterns of Tb while febrile resulted in a less pronounced 24-hour rhythm. Inducing fever at different times of the day in different individuals of the same species may provide further evidence that LPS does in fact disrupt the normal circadian variations in Tb. In addition, LPS-induced fever needs to be investigated in other marsupials species as it is highly likely that some species may have more lengthy fevers than *M.*

domestica. This intraspecies difference has previously been observed in rodents (eg Conrad et al., 1997).

It has previously been proposed that the hyperthermic response seen in LPS-induced fever is due to the shifting of the thermoregulatory set-point. As the *P. breviceps* selected warmer environmental temperatures during LPS fever, it is apparent that such a process may occur. However, such thermoregulatory behaviour was not observed in *M. domestica*. This is most likely due to *M. domestica* being a laboratory-bred animal maintained in a constant environment unlike the captive *P. breviceps*. Muchlinski et al., (2000) found differences between laboratory-maintained and free-living ground squirrels with respect to fever as the free-living animals failed to produce a fever in response to *E. coli* LPS. According to these authors this was due to a pre-existing elevation in thermoregulatory set-point in the free-living animals which resulted in the maintenance of a higher mean Tb in these animals. It would be interesting to conduct behavioural thermoregulation and fever experiments on free-living *M. domestica* and see if they behaviourally thermoregulate when febrile.

In eutherian mammals, the magnitude of fever is believed to be determined by endogenous pyrogens and antipyretics acting at the central nervous system (eg Kluger, 1991). PG, in particular, is known to influence the hypothalamic response to LPS (eg Fraifeld et al., 2000). Such compounds have not been investigated in marsupial species and so their particular role in marsupial fever is unknown. However, as marsupials exhibit a LPS fever comparable to eutherians it is proposed that the mechanisms of fever (ie role of eicosanoids such as PG) are common in all mammals. A recent study by Fraifeld et al., (2001) has also provided evidence that such eicosanoids are involved in the regulation of normal daily variations of Tb in rats and mice. This further highlights the need to investigate the role of PG and similar compounds in marsupial thermoregulation and fever. As the fever response in *M. domestica* is

comparable to eutherian species it is highly likely that the endogenous mediators of fever are also similar.

Hypothermic responses to hypoxia are commonly observed in small mammalian species (eg Frappell et al., 1992) and hypoxia depresses core Tb throughout a full 24-hour circadian cycle in small non-macropod marsupials such as *M. domestica*. Upon return to normoxia, normal Tb rhythms are resumed after an initial hyperthermic increase in core Tb. Hypothermia induced by hypoxia results from a decreased heat production while simultaneously increasing heat loss. This is advantageous as it lowers metabolic rate and thus oxygen requirements thereby enhancing survival. Little is known however about the underlying mechanisms by which hypoxia suppresses thermogenesis.

The mechanisms of hypothermia in lower vertebrates are primarily behavioural (ie animals seek cooler environments) with a physiological component of peripheral blood flow. In mammals, the mechanisms of hypothermia are not completely understood but are likely to involve both autonomic and behavioural effectors. The difficulties in understanding mammalian hypothermia are partly due to the close relationship between metabolic rate and Tb regulation and the difficulty in determining whether a decreased Tb is a cause or effect of a reduced metabolic rate. In mammals, interactions between hypoxia and hypothermia are further complicated by thermogenic responses to reduced Tb. The effects of hypoxia on thermoregulation and metabolism are more evident in small animals as they have lower thermogenic requirements to maintain endothermy. Hypoxia disrupted normal circadian rhythms of Tb in *M. domestica* with rhythms of Tb ranging from 12 to 48 hours observed. In the one *P. breviceps* studied the circadian rhythm of Tb was reduced from 24 hours to less than 12 hours when the animal was exposed to hypoxia. This may reflect a change in the hypothalamic set-point of thermoregulation by hypoxia in this animal. Such a change in hypothermic set-point is not evident in the laboratory-bred *M. domestica* as three animals of this species maintained a 24-hour rhythm of Tb

during hypoxia although the rhythm was not as pronounced as that seen during normoxia.

The use of behavioural thermoregulation during hypoxia has rarely been considered in endotherms although it is relatively well documented in ectothermic species (eg Greishaber et al., 1994). Behavioural thermoregulation was not readily utilised by *M. domestica* during exposure to hypoxia indicating that this laboratory-bred marsupial species used only autonomic mechanisms to reduce Tb. However, the one *P. breviceps* did take advantage of the range of Tas available in the thermal gradient when exposed to hypoxic conditions which likely contributed to this animal's altered circadian rhythm of Tb. This latter, captive animal, selected cooler Tas while inactive and exposed to hypoxia but exhibited no circadian rhythm of Ta selection during hypoxia. This is not unusual for an endotherm as observed by Gordon (1994) in rats, although opposing rhythms of behavioural thermoregulation have been observed in hamsters (Refinetti, 1995b) and humans (Shoemaker and Refinetti, 1996). As behaviour was not similarly observed in any of the laboratory-bred *M. domestica* studied it is evident that behaviour is a thermoregulatory tool that can be learnt by marsupials when they are bred and maintained in a variable (natural) environment. Using laboratory-bred animals for thermoregulatory studies involving behaviour therefore may be limiting with respect to the overall thermoregulatory strategies utilised by the species in its natural environment.

Although few studies have previously investigated the interactions between hypoxia and fever and their simultaneous effects on thermoregulation it is apparent that an interaction does occur through the modulation of the thermoregulatory system. As shown in this study, hypoxia can blunt the typical hyperthermic response of LPS-induced fever in mammals including marsupials as seen in *M. domestica*. The ability of hypoxia to do this however can be modulated or inhibited through

behavioural selection of higher Tas as seen in the *P. breviceps*. This may also be the case in other marsupial species.

Circadian rhythms of Tb were affected by a combination of LPS and hypoxia in two *M. domestica* in this study while the other three *M. domestica* and the *P. breviceps* maintained a 24-hour rhythm of Tb. This general lack of disruption to circadian patterns of Tb could be related to the short exposure to LPS and hypoxia and the timing of this exposure which was always in the afternoon hours. Interestingly, the two animals that did not maintain a 24-hour rhythm during LPS and hypoxia both exhibited a minimal change in Tb during the afternoon when exposed to only hypoxia. In addition, one animal exhibited a 12-hour Tb cycle during hypoxia while the other exhibited a 48-hour rhythm of Tb during fever. Whether these differences are typical of individual animals or whether they are a reflection of their captivity bred status is unknown and requires further investigation of both laboratory-bred and free-living or captive species. The maintenance of a 24-hour rhythm of Tb in the majority of animals does indicate though that the hypothalamic set-point is shifted during LPS and hypoxia in *M. domestica*.

Like other endotherms such as rats and guinea pigs, *M. domestica* suppresses hyperthermic responses to LPS when exposed to hypoxia. Presumably like *M. domestica* in this study, these previous experiments (eg Almeida et al., 1999) used laboratory bred animals and therefore may not be a true representation of thermoregulatory responses to LPS and hypoxia in these species. This is particularly apparent as the captive *P. breviceps* used in this study responded very differently. This animal increased Tb in response to a combination of LPS and hypoxia with concurrent selection of higher Tas during exposure (to LPS and hypoxia). This behavioural thermoregulation made a major contribution to the degree of hyperthermia observed in this animal. Again this highlights the possible importance of the environment in thermoregulation in free-living animals and the unforeseen inadequacies of the traditional use of laboratory animals, particularly in investigations of behavioural thermoregulation.

This study has been restricted by the availability of animals; *M. domestica* availability was restricted due to quarantine reasons (as it is a South American species and the study was conducted in Australia) and only one *P. breviceps* was used in the study despite numerous attempts to capture more animals of this species (as outlined in the preface of this thesis). Consequently data obtained from the *P. breviceps* could only be used as a comparison to the general results observed in *M. domestica.* Such a comparison did highlight some interesting observations from the *P. breviceps* which remain inconclusive at this stage.

The general thermoregulatory responses to LPS and hypoxia and normal circadian rhythms of core Tb were similar in *M. domestica* and the *P. breviceps* despite the small numbers used. However, responses to a combination of LPS and hypoxia and the use of behavioural thermoregulation did differ. It is most probable that these differences can be attributed to the knowledge that the *M. domestica* used were laboratory-bred (and maintained at a strictly regulated Ta and day-night cycle) while the single *P. breviceps* was obtained from its natural environment. Such profound differences between the thermal performances of captive-bred and free-living feathertail gliders have also been reported by Geiser and Ferguson (2001). Regardless of the reasoning behind these differences this study has highlighted a number of important aspects of marsupial thermoregulation.

Firstly, laboratory-bred *M. domestica* exhibit circadian patterns of Tb within a 24-hour cycle typical of most mammals. These patterns of Tb are affected by ambient temperature with cold ambient conditions disrupting normal circadian rhythms. Secondly, behavioural thermoregulation has little circadian basis in *M. domestica* but is seen to modulate thermoregulation as a means of saving energy. Thirdly, this study indicates that a non-macropod marsupial, *M. domestica* does display a typical hyperthermic mammalian fever to bacterial LPS and a typical hypothermic

and hypometabolic response to hypoxia. Fourthly, fever responses in *M. domestica* may be suppressed by hypoxia although thermoregulatory behaviour may overcome this effect on core Tb. Finally, a laboratory-bred non-macropod marsupial species (*M. domestica*) does not respond to fever and hypoxia in a way that supports the theory of the set-point of thermoregulation.

The study certainly highlights the need to consider the natural habitat conditions of a species when investigating thermoregulation, particularly behavioural thermoregulation. Many studies on thermoregulation are based on laboratory animals which do not necessarily reflect the natural thermoregulatory capacities of a species. Traditional laboratory experiments indicate the mechanisms (particularly the autonomic mechanisms) of thermogenesis, but field studies would provide a more sound indication of the thermoregulatory strategies (particularly behavioural) utilised by the animal. This has also been suggested by Mitchell et al., (unpublished review) who suggest that observations in circadian rhythms of Tb in large arid-zone mammals result primarily from experimental constraints which prevent animals from utilising thermoregulatory effectors, particularly thermoregulatory behaviour. In addition, as mentioned previously, Geiser and Ferguson (2001) have reported differences between patterns of torpor in captive and free-living feathertail gliders (*A. pygmaeus*) and free-living *P. breviceps* exhibit longer, deeper and more frequent bouts of torpor than that observed in the laboratory (Kortner and Geiser, 2000). These authors show that breeding animals in captivity or using animals acclimated to a captive environment strongly affects both behaviour and physiology.

It would be of particular interest to repeat experiments on thermoregulatory behaviour, fever and hypoxia on free-living *M. domestica* and compare the data to that obtained in this study. Furthermore, similar experiments need to be performed on other marsupial species, where possible, using animals bred and maintained in their natural surroundings.

This study therefore clearly supports the need and importance for field studies to be conducted in thermoregulation of marsupials to validate a number of theories proposed by observations in laboratory animals.

In order to understand the full mechanisms of thermogenesis, including the concept of the set-point theory of thermoregulation, experiments need to move away from the laboratory environment and into the natural habitats. This is the case for both ectothermic and endothermic animals. Only then will we fully understand thermoregulation in all animals especially mammals such as marsupials. This will allow us to make more reliable comparisons between individual species and to fully understand the thermoregulatory capabilities of species throughout the animal kingdom.

CHAPTER 11

REFERENCES

Abramovich, I.; Fraifield, V.; Shemi, D. and Kaplanski, J. (1994). Effect of LPS and indomethacin on short term fever response and hypothalamic PGE_2 production in mice. *Can. J. Physiol. Pharmacol.* 72 (Suppl. 1): 286.

Adair, E.R. (1976). Autonomic thermoregulation in the squirrel monkey when behavioural regulation is limited. *J. Appl. Physiol.* 40: 694-700.

Adamo, S.A. (1998). The specificity of behavioural fever in the cricket, *Acheta domesticus. J. Parasitol.* 84: 529-533.

Akins, C. and Thiessen, D.D. (1990). Lipopolysaccharide decreases ambient temperature preference in Mongolian gerbils, *Meriones unguiculatus. Percept. Mot. Skills* 71: 1177-1178.

Akins, C.; Thiessen, D. and Cocke, R. (1991). Lipopolysaccharide increases ambient temperature preference in C57BL/6J adult mice. *Physiol. Behav.* 50: 461-463.

Almeida, M.C.; Carnio, E.C. and Branco, L.G. (1999). Role of nitric oxide in hypoxia inhibition of fever. *J. Appl. Physiol.* 87: 2186-2190.

Amini-Sereshki-Kormi, L. (1996). Effects of ambient temperature on thermoregulation during fever in cats. *Pathobiology* 64(5): 284-288.

Arancibia, S.; Rage, F.; Astier, H. and Tapia-Arancibia, L. (1996). Neuroendocrine and autonomous mechanisms underlying thermoregulation in cold environment. *Neuroendocrinology* 64: 257-267.

Arya, G. and Garcia, V.F. (1995). Hypoxia/Reoxygenation affects endotoxin tolerance. *J. Surg. Res.* 59: 13-16.

Aschoff, J. (1966). Circadian activity patterns with two peaks. *Ecology* 47: 657-662.

Aschoff, J. (1970). Circadian rhythm of activity and body temperature. In: *Physiological and Behavioural Temperature Regulation.* Eds. J.D. Hardy, A.P. Gagge and J.A.J. Stolwijk. Charles C. Thomas, Springfield, Illinois. p. 905-919.

Aschoff, J. (1982). The circadian rhythm of body temperature as a function of body size. In: *A Companion to Animal Physiology.* Eds. C.R. Taylor; R. Johansen and L. Bolis. Cambridge University Press, Cambridge. p. 173-188.

Baldwin, B.A. (1968). Behavioural thermoregulation in mice. *Physiol. Behav.* 3: 401-407.

Banin, D.; Haim, A. and Arad, Z. (1994). Metabolism and thermoregulation in the levant vole *Microtus guentheri*: The role of photoperiodicity. *J. Therm. Biol.* 19: 55-62.

Bartholomew, G.A. and Hudson, J.W. (1962). Hibernation, estivation, temperature regulation, evaporative water loss and heart rate of the pigmy possum *Cercetetus nanus. Physiol. Zool.* 35: 94-107.

Barzel, U.S. (1989). Thyroid hormones: Blood levels do not define status of metabolism [letter]. *J. Am. Geriatr. Soc.* 37: 91.

Baudinette, R.V. (1984). Thermoregulation in marsupials during exercise. In: *Thermal Physiology* Ed. J.R.S. Hales. Raven, New York. p.423-427.

Beasley, L.J. and Nelson, R.J. (1982). Thyroid gland influences the period of hamster circadian oscillations. *Experentia* 38: 870-871.

Bencsics, A.; Elenkov, I.J.; Vizi, E.S. (1995). α_2-, α_{2A}-, $\alpha_{2B/2C}$-Adrenoceptor subtype antagonists prevent lipopolysaccharide-induced fever response in rabbits. *Brain Res.* 705: 302-306.

Bender, B.S.; Cottey, R.; Bell, W. and Taylor, S. (1996). Body temperature and nesting behaviour following influenza challenge in mice: Effects of age. *Mechanisms of Ageing and Development* 86: 1-9.

Bennett, A.F. (1991). The evolution of aerobic capacity. *J. Exp. Biol.* 160: 1-23.

Berk, M.L. and Heath, J.E. (1975). An analysis of behavioural thermoregulation in the lizard, *Dipsosaurus dorsalis. J. Therm. Biol.* 1:56-78.

Binkley, S.; Kluth, E. and Menaker, M. (1971). Pineal function in sparrows: Circadian rhythms and body temperature. *Science* 174: 311-314.

Blatteis, C.M. (1983). Thermal responses of hamsters to endotoxin. *J. Therm. Biol.* 8: 195-197.

Blatteis, C.M. and Lutherer, L.O. (1973). Cold-induced thermogenesis in dogs: its reduction by moderate hypoxia. *J. Appl. Physiol.* 39: 251-257.

Blatteis, C.M. and Smith, K.A. (1980). Behavioural fever in guinea-pigs by intra-preoptic pyrogen. *Experentia* 36: 1086-1088.

Bligh, J. (1966). The thermosensitivity of the hypothalamus and thermoregulation in mammals. *Biol. Rev.* 41: 317-367.

Bligh, J. and Harthoorn, A.M. (1965). Continuous radiotelemetric records of the deep body temperature of some unrestrained African mammals under near natural conditions. *J. Physiol.* 176: 145-162.

Boorstein, S.M. and Ewald, P.W. (1987). Costs and benefits of behavioural fever in *Melanoplus sanguinipes* infected by *Nosema acridophagus*. *Physiol. Zool.* 60: 586-595.

Boutilier, R.G.; West, T.G.; Webber, D.M.; Pogson, G.H.; Mesa, K.A.; Wells, J. and Wells, M.J. (2000). The protective effects of hypoxia-induced hypometabolism in the Nautilus. *J. Comp. Physiol. B.* 170: 261-268.

Bowker, R.G. (1984). Precision of thermoregulation in some African lizards. *Physiol. Zool.* 57: 401-412.

Branco, L.G.; Carnio, E.C. and Barros, R.C. (1997). Role of the nitric oxide pathway in hypoxia-induced hypothermia of rats. *Am. J. Physiol.* 273: R967-R971.

Branco, L. and Wood, S.C. (1994). Role of central chemoreceptors in behavioural thermoregulation of the toad. *Am. J. Physiol.* 266: R1483-R1487.

Brauer, R.W.; Johnson, E.D.; Miller, C.G.; Sanchez-Grinan, M.I.; Shelton, M.G. and Williams, E.E. (1986). Modification of temperature preference behaviour by hypoxia and metabolic inhibitors. In: *Homeostasis and Thermal Stress: Experimental and therapeutic advances.* Eds. K. Cooper; P. Lomax; E. Schonbaum, E. and W. L. Veale. 6th Int. Symp. Pharm. Thermoregulation. Karger, Basel. p. 27-29.

Brauer, R.W.; Jordan, M.R.; Kinney, M.; Miller, G. and Williams, E.E. (1984). Temperature/Pressure interactions in regard to temperature and pressure preference behaviour. In: *Thermal Physiology* Ed. J.R.S. Hales. Raven, New York. p. 499-502.

Briese, E. (1985). Rats prefer ambient temperatures out of phase with their body temperature circadian rhythm. *Brain Res.* 345: 389-393.

Briese, E. (1986). Circadian body temperature rhythm and behaviour of rats in thermoclines. *Physiol. Behav.* 37: 839-847.

Briese, E. and de Quijada, M.G. (1970). Colonic temperature of rats during handling. *Acta Phsiol. Latinoam.* 20: 97-102.

Bronstein, S.M. and Connor, W.E. (1984). Endotoxin-induced behavioural fever in the Madagascar cockroach, *Gromphadorhina portentosa*. *J. Insect Physiol.* 30: 327-330.

Brown, G.D. and Dawson, T.J. (1977). Seasonal variations in the body temperatures of unrestrained kangaroos (*Macropodidae marsupialia*). *Comp. Biochem. Physiol.* 56A: 59-67.

Brown, C.M. and Refinetti, R. (1996). Daily rhythms of metabolic heat production, body temperature and locomotor activity in golden hamsters. *J. Therm. Biol.* 21: 227-230.

Budgell, P. (1971). Behavioural thermoregulation in the Barbary dove (*Streptopelia risoria*). *Anim. Behav.* 19: 524-531.

Cabanac, M. (1990). Phylogeny of fever. In: *Thermoreception and Temperature Regulation.* Eds. J. Bligh and K. Voigt. Springer-Verlag, Berlin. pp. 284-296.

Cabanac, A. and Briese, E. (1991). Handling elevates the colonic temperature of mice. *Physiol. Behav.* 51: 95-98.

Cabanac, A. and Gosselin, F. (1993). Emotional fever in the lizard *Callopistes maculatus*. *Anim. Behav.* 46: 200-202.

Cabanac, M.; Duclaux, R. and Gillet, A. (1970). Thermoregulation compartmentale chez le chien: Effects de la fievre et de la thyroxine. *Physiol. Behav.* 5: 697-704..

Cabanac, M.; Hildebrandt, G.; Massonet, B. and Strempel, H. (1976). A study of the nycthermal cycle of behavioural temperature regulation in man. *J. Physiol.* 257: 275-291.

Cabanac, M. and Laberge, F. (1998). Fever in goldfish is induced by pyrogens but not by handling. *Physiol. Behav.* 63: 377-379.

Cabanac, M. and Massonet, B. (1974). Temperature regulation during fever: Change of set point or change of gain? A tentative answer from a behavioural study in man. *J. Physiol.* 238: 561-568.

Cabanac, M. and Rossetti, Y. (1987). Fever in snails, reflection on a negative result. *Comp. Biochem. Physiol.* 87A: 1017-1020.

Campbell, J.D. (1985). The influence of metabolic cost upon the level and precision of behavioural thermoregulation in an eurhythmic lizard. *Comp. Biochem. Physiol.* 81A: 597-601.

Canals, M.; Rosenmann, M. and Bozinovic, F. (1989). Energetics and geometry of huddling in small mammals. *J. Theor. Biol.* 141: 181-189.

Cheymol, J. and Levasort, C. (1955). Actions comparaees de l'hypoxie et de la clorpormazine sur differents types d'hyperthermies. *Annales Pharmaceutiques Francaises (Paris)* 13: 527-538.

Chossat, Ch. (1843). Recherches experimentales sur l'inanition sur la chaleur animale. *Ann. Sci. Naturelles 2 Serie* 20: 293-326.

Clark, D.J. and Fewell, J.E. (1996). Decreased body-core temperature during acute hypoxemia in guinea pigs during postnatal maturation: a regulated thermoregulatory response. *Can. J. Physiol. Pharmacol.* 74: 331-336.

Clark, S.M.; Gean, J.T. and Lipton, J.M. (1980). Reduced febrile responses to peripheral and central administration of pyrogen in aged squirrel monkeys. *Neurobiol. Age* 1: 175-180.

Clark, W.G. and Lipton, J.M. (1974). Complementary lowering of the behavioural and physiological thermoregulatory set-points by tetrodotoxin and saxitoxin in the cat. *J. Physiol.* 238: 181-191.

Coelho, M.M.; Souza, G.E.P. and Pela, I.R. (1992). Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. *Am. J. Physiol.* 263: R423-R427.

Cokelaere, M.; Decuypere, E.; Flo, G.; Darras, V.M. and Kuhn, E.R. (1996). Influence of feeding pattern on thyroid hormones in long-term food-restricted rats. *Horm. Metab. Res.* 28: 315-318.

Collins, K.J.; Dore, C.; Exton-Smith, A.N.; Fox, R.H.; McDonald, I.C. and Woodward, P.M. (1977). Accidental hypothermia and impaired temperature homeostasis in the elderly. *Br. Med. J.* 1: 353-356.

Collins, K.J.; Exton-Smith, A.N. and Dore, C. (1981). Urban hypothermia: Preferred temperature and thermal perception in old age. *Br. Med. J.* 282: 175-177.

Conn, C.A.; Borer, K.T. and Kluger, M.J. (1990). Body temperature rhythm and response to pyrogen in exercising and sedentary hamsters. *Med. Sci. Sports Ex.* 22: 636-642.

Connor, D.G. and Kass, E.H. (1961). Effect of artificial fever in increasing susceptibility to bacterial endotoxin. *Nature* 190: 453-454.

Conrad, A.; Bull, D.F.; King, M.G. and Husband, A.J. (1997). The effects of lipopolysaccharide (LPS) on the fever response in rats at different ambient temperatures. *Physiol. Behav.* 62: 1197-1201.

Cooper, K.E. (1987). The neurobiology of fever: Thoughts on recent developments. *Ann. Rev. Neurosci.* 10: 297-324.

Corbit, J.D. (1970). Behavioural regulation of body temperature. In: *Physiological and Behavioural Temperature Regulation.* Eds. J.D. Hardy; A.P. Gagge and J.A.J. Stolwijk. C. C. Thomas, Springfield, Illinois. p. 777-801.

Cortes, A.; Baez, C.; Rosenmann, M. and Pino, C. (1994). Body temperature, activity cycle and metabolic rate in a small, nocturnal Chilean lizard, *Garthia gaudichaudi* (Sauria: Geldcondiae). *Comp. Biochem. Physiol.* 109: 967-973.

Côté, I.M.; Reverdy, B. and Cooke, P.K. (1998). Less choosy or different preference? Impact of hypoxia on hermit crab shell assessment and selection. *Anim. Behav.* 56: 867-873.

Cowgell, J. and Underwood, H. (1979). Behavioural thermoregulation in lizards: A circadian rhythm. *J. Exp. Zool.* 210: 189-194.

Crawshaw, L.I. and Stitt, J.T. (1975). Behavioural and autonomic induction of prostaglandin E_1 fever in squirrel monkeys. *J. Physiol.* 244: 197-206.

Crompton, A.W.; Taylor, C.R. and Jager, J.A. (1978). Evolution of homeothermy in mammals. *Nature* 272: 333-336.

Cronin, L. (1991). *Key guide to Australian marsupials.* Reed Books, Balgowlah, NSW.

D'Alecy, L.G. and Kluger, M.J. (1975). Avian febrile response. *J. Physiol.* 253: 223-232.

Dauncey, M.J. (1990). Thyroid hormones and thermogenesis. *Proc. Nutr. Soc.* 49: 203-215.

Dawson, T.J. (1973). Primitive mammals. In: *Comparative Physiology of Thermoregulation.* Ed. C.G. Whittow. Vol. 3. Academic Press, New York.

Dawson, T.J. (1984). Unusual features in thermoregulatory effector systems of marsupials. In: *Thermal Physiology* Ed. J.R.S. Hales. Raven, New York. p. 289-294.

Dawson, T.J. and Dawson, W.R. (1982). Metabolic scope and conductance in response to cold of some dasyurid marsupials and Australian rodents. *Comp. Biochem. Physiol.* 71A: 59-64.

Dawson, T.J.; Denny, M.J.S. and Hulbert, A.J. (1969). Thermal balance of the macropodid marsupial *Macropus eugenii* desmarest. *Comp. Biochem. Physiol.* 31: 645-653.

Dawson, T.J. and Hulbert, A.J. (1970). Standard metabolism, body temperature, and surface areas of Australian marsupials. *Am. J. Physiol.* 218: 1233-1238.

Dawson, T.J. and May, E.L. (1984). Daily variation in brain and body temperatures of the sugar glider (*Petaurus breviceps*): Some insights into the control of thermoregulation. In: *Possums and Gliders* Eds A.P. Smith and I.D. Hume, Australian Mammal Society, Sydney. p. 375- 383.

Dawson, T.J. and Olson, J.M. (1988). Thermogenic capabilities of the opossum, *Monodelphis domestica*, when warm and cold acclimated: similarities between American and Australian marsupials. *Comp. Biochem. Physiol.* 89A: 85-91.

DeCoursey, P.J.; Pius, S.; Sandlin, C.; Wethey, D. and Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiol. Behav.* 65: 457-463.

Deen, C.M. and Hutchison, V.H. (2001). Effects of lipopolysaccharide and acclimation temperature on induced behavioural fever in juvenile *Iguana iguana. J. Therm. Biol.* 26: 55-63.

DeRijk, R.H.; Van Kampen, M.; Van Rooijen, N. and Berkenbosch, F. (1994). Hypothermia to endotoxin involves reduced thermogenesis, macrophage-dependent mechanisms, and prostaglandins. *Am. J. Physiol.* 266: R1-R8.

Dicker, A.; Cannon, B. and Nedergaard, J. (1995). Cold acclimation-recruited nonshivering thermogenesis: the Syrian hamster is an exception. *Am. J. Physiol.* 269: R767-R774.

Doherty, Jr D.W. and Blatteis, C.M. (1980). Hypoxic reduction of endotoxic fever in guinea pigs. *J. Appl. Physiol. Resp. Environ. Exercise Physiol.* 49(2): 294-299.

Douglas, T.A. (1990). The water regulatory efficiency of Australian marsupials. B.Sc. (Hons) Thesis, Department of Biological Sciences, Flinders University of South Australia.

Dubois-Ferrière, R. and Chinet, A.E. (1981). Contribution of skeletal muscle to the regulation of NST in small mammals. *Pfluegers Arch.* 390: 224-229.

Duchamp, C. and Barre, H. (1993). Skeletal muscle as the major site of nonshivering thermogenesis in cold-acclimated ducklings. *Am. J. Physiol.* 265: B1076-B1083.

Duprè, R.K. and Owen, T.L. (1989). Behavioural thermoregulation in hypoxic rats. *FASEB J.* 3: A838.

Duprè, R.K. and Owen, T.L. (1992). Behavioural thermoregulation by hypoxic rats. *J. Exp. Zool.* 262: 230-235.

Duprè, R.K.; Romero, A.M. and Wood, S.C. (1988). Thermoregulation and metabolism in hypoxic animals. In: *Oxygen transfer from atmosphere to tissues. Advances in Experimental Medicine and Biology*. Vol. 227. Eds. N.C. Gondalez and M.R. Fedde. Plenum Press, New York. p. 347-351.

Duprè, R.K. and Wood, S.C. (1988). Behavioural temperature regulation by aquatic ectotherms during hypoxia. *Can J. Zool.* 66: 2649-2652.

Eayrs, J.T. (1966). Thyroid and central nervous development. *Sci. Basis Med. Annu. Rev.*: 317-319.

Edelman, I.S. (1974). Thyroid thermogenesis. *N. Engl. J. Med.* 290: 1303-1308.

Eedy, J.W. and Ogilvie, D.M. (1970). The effect of age on the thermal preference of white mice and gerbils. *Can. J. Zool.* 48: 1303-1306.

Evans, S.E. and Ingram, D.L. (1974). The significance of deep body temperature in regulating the concentration of thyroxine in the plasma of the pig. *J. Physiol.* 236: 159-170.

Fadem, B.H.; Trupin, G.L.; Maliniak, E.; VandeBerg, J.L. and Hayssen, V. (1982). Care and breeding of the grey short-tailed opossum (*Monodelphis domestica*). *Lab. Anim. Sci.* 32: 405-409.

Farkas, M.; Mozsa, S. and Donhoffer, S. (1966). The effect of hypoxic hypoxia and environmental temperature on body temperature and oxygen consumption in the course of pyrogen-induced fever. *Acta Physiol. Acad. Sci. Hung.* 30: 155-160.

Feldberg, W. and Gupta, K.P. (1973). Pyrogen fever and prostaglandin like activity in cerebrospinal fluid. *J. Physiol.* 228: 41-53.

Feldberg, W. and Saxena, P.N. (1975). Prostaglandins, endotoxin and lipid A on body temperature in rats. *J. Physiol.* 249: 601-615.

Ferguson, A.V.; Bauce, L. and Cooper, K.E. (1983). An investigation of age-related deficit in the febrile response of the rabbit. *Am. J. Physiol.* 245: R379-R385.

Ferguson, A.V.; Veale, W.L. and Cooper, K.E. (1981). Age-related differences of the New Zealand White rabbit to endotoxin. *Can. J. Physiol. Pharmacol.* 59: 613-614.

Firth, B.T.; Ralph, C.L. and Boardman, T.J. (1980). Independent effects of the pineal and a bacterial pyrogen in behavioural thermoregulation in lizards. *Nature* 285: 399-400.

Fisher, D.A. (1996). Physiological variations in thyroid hormones: physiological and pathological considerations. *Clin. Chem.* 42: 135-139.

Fisher, D.A.; Dussault, J.H.; Sack, J. and Chopra, I.J. (1977). Ontogenesis of hypothalamic-pituitary-thyroid function and metabolism in man, sheep, and rat. *Recent Prog. Horm. Res.* 33: 59-116.

Flannery, T.F. (1994). *Possums of the World. Monograph of the Phalangeroidea.* Geo Productions, Sydney.

Fleming, M.R. (1980). Thermoregulation and torpor in the sugar glider, *Petaurus breviceps* (Marsupialia: Petauridae). *Aust. J. Zool.* 28: 521-534.

Florez-Duquet, M. and McDonald, R.B. (1998). Cold-induced thermoregulation and biological aging. *Physiol. Rev.* 78: 339-358.

Florez-Duquet, M.; Peloso, E. and Satinoff, E. (2001). Fever and behavioural thermoregulation in young and old rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280: R1457-1461.

Ford, D.H. (1968). Central nervous system-thyroid interrelationships. *Brain Res.* 7: 329-349.

Fraifeld, V.; Abramovich, L. and Kaplanski, J. (1995). Delayed febrile response in old rats is not associated with an inability of hypothalamus to produce prostaglandin E_2. *Mech. Ageing Dev.* 79: 137-140.

Fraifeld, V. and Kaplanski, J. (1998). Brain eicosanoids and LPS fever: species and age differences. In: *Progress in Brain Research.* Eds. H.S. Sharma and J. Westman. Vol. 115. Elsevier Science. p. 141-157.

Fraifeld, V.; Paul, L. and Kaplanski, J. (2000). The relationship between hypothalamic prostaglandin E_2 or leukotrienes and the body temperature response to lipopolysaccharide in different murine strains. *J. Therm. Biol.* 25: 17-20.

Fraifeld, V.; Paul, L.; Kaplanski, J.; Kozak, W. and Kluger, M.J. (2001). Evidence for the involvement of eicosanoids in the regulation of normal body temperature. *J. Therm. Biol.* 26: 295-297.

Francis, A.J.P. and Coleman, G.J. (1997). Phase response curves to ambient temperature pulses in rats. *Physiol. Behav.* 62: 1211-1217.

Frappell, P.B.; Franklin, C.E. and Grigg, G.C. (1994). Ventilatory and metabolic responses to hypoxia in the echidna, *Tachyglossus aculeatus*. *Am. J. Physiol.* 267: R1510-R1515.

Frappell, P.B.; Lanthier, C.; Baudinette, R.V. and Mortola, J.P. (1992). Metabolism and ventilation in acute hypoxia: a comparative analysis in small mammalian species. *Am. J. Physiol.* 262: R1040-R1046.

Freake, H.C. and Oppenheimer, J.H. (1995). Thermogenesis and thyroid function. *Annu. Rev. Nutr.* 15: 263-291.

Frey, H. and Fleming, M.R. (1984). Torpor and thermoregulatory behaviour in free-ranging feathertail gliders (*Acrobates pygmaeus*) (Marsupialia: Burramyidae) in Victoria. In: *Possums and Gliders.* Eds. A.P. Smith and I.D. Hume, Australian Mammal Society, Sydney.

Fukuhara, K.; Kvetnansky, R.; Cizza, G.; Pacak, K.; Ohara, H.; Goldstein, D.S. and Kopin, I.J. (1996). Interrelations between sympatho-adrenal system and hypothalamo-pituitary-adrenocortical/thyroid systems in rats exposed to cold stress. *J. Neuroendocrinol.* 8: 533-541.

Fuller, C.A. (1984). Circadian brain and body temperature rhythms in the squirrel monkey. *Am. J. Physiol.* 246: R242-R246.

Fuller, C.A. and Sulzman, F.M. (1982). Circadian control of body temperature in primates. In: *Vertebrate Circadian Rhythms.* Eds. J. Aschoff; S. Daan and G. Groos. Springer-Verlag, Berlin. pp. 224-236.

Galton, V.A. (1983). Thyroid hormone action in amphibian metamorphosis. In: *Molecular Basis of Thyroid Hormone Action.* Eds. J.H. Oppenheimer and H.H. Samuels. Academic Press, New York. pp. 445-483.

Gautier, H. (1996). Interactions among metabolic rate, hypoxia and control of breathing. *J. Appl. Physiol.* 81: 521-527.

Gautier, H.; Bonora, M.; Ben M'Barek, S. and Sinclair, J.D. (1991). Effects of hypoxia and cold acclimation on thermoregulation in the rat. *J. Appl. Physiol.* 71(4): 1355-1363.

Gautier, H.; Bonora, M.; Schultz, S.A. and Remmers, J.E. (1987). Hypoxia-induced changes in shivering and body temperature. *J. Appl. Physiol.* 62: 2477-2484.

Geiser, F. (1986). Temperature regulation in heterothermic marsupials. In: *Living in the Cold: Physiological and Biochemical Adaptations.* Eds. H.C. Heller, X.J. Musacchia and L.C.H. Wang. Elsevier Science Publishing, New York.

Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *J. Comp. Physiol. B* 158: 25-37.

Geiser, F. (1994). Hibernation and daily torpor in marsupials: a review. *Aust. J. Zool.* 42: 1-16.

Geiser, F. and Ferguson, C. (2001). Intraspecific differences in behaviour and physiology: effects of captive breeding on patterns of torpor in feathertail gliders. *J. Comp. Physiol. B.* 171: 569-576.

Geiser, F. and Masters, P. (1994). Torpor in relation to reproduction in the mulgara, *Dasycercus cristicauda* (Dasyuridae: Marsupialia). *J. Therm. Biol.* 19: 33-40.

Gellhorn, E. and Janus, A, (1936), The influence of partial pressures of O_2 on body temperature. *Am. J. Physiol.* 116: 327-329.

Gemmell, R.T. and Cepon, G. (1993). The development of thermoregulation in the marsupial brushtail possum *Trichosurus vulpecula. Comp. Biochem. Physiol.* 106A: 167-173.

Gemmell, R.T.; Cepon, G. and Barnes, A. (1987). The development of thermoregulation in the rat kangaroo, *Potorous tridactylus. Comp. Biochem. Physiol.* 88A: 257-261.

Gemmell, R.T. and Johnston, G. (1985). The development of thermoregulation and emergence from the pouch of the marsupial bandicoot, *Isoodon macrourus. Physiol. Zool.* 58: 299-302.

George, J.C. (1984). Thermogenesis in birds. In: *Thermal Physiology* Ed. J.R.S. Hales. Raven, New York. p. 467-473.

Goelst, K. and Laburn, H. (1991). Response of body temperature and serum iron concentration to repeated pyrogen injection in rabbits. *Pflugers Arch.* 417: 558-561.

Gordon, C.J. (1983a). A review of terms for regulated vs. forced neurochemical-induced changes in body temperature. *Life Sci.* 32: 1285-1295.

Gordon, C.J. (1983b). Behavioural and autonomic thermoregulation in mice exposed to microwave radiation. *J. Appl. Physiol.* 55: 1242-1248.

Gordon, C.J. (1985). Relationship between autonomic and behavioural thermoregulation in the mouse. *Physiol. Behav.* 34: 687-690.

Gordon, C.J. (1986). Relationship between behavioural and autonomic thermoregulation in the guinea pig. *Physiol. Behav.* 38: 827-831.

Gordon, C.J. (1987). Relationship between preferred ambient temperature and autonomic thermoregulatory function in the rat. *Am. J. Physiol.* 252: R1130-R1137.

Gordon, C.J. (1988). Simultaneous measurement of preferred ambient temperature and metabolism in rats. *Am. J. Physiol.* 254: R229-R234.

Gordon, C.J. (1993a). Twenty-four hour rhythms of selected ambient temperature in the rat and hamster. *Physiol. Behav.* 53: 257-263.

Gordon, C.J. (1993b). *Temperature Regulation in Rodents.* Cambridge University Press, New York.

Gordon, C.J. (1994). Twenty-four hour control of body temperature in rats I. Integration of behavioural and autonomic effectors. *Am. J. Physiol.* 267: R171-R177.

Gordon, C.J.; Baker Bailey, E.; Kozel, W.M. and Ward, G.H. (1983). A device for monitoring position of unrestrained animals in a temperature gradient. *Physiol. Behav.* 31: 265-268.

Gordon, C.J.; Fehlner, K.S. and Long, M.D. (1986). Relationship between autonomic and behavioural thermoregulation in the golden hamster. *Am. J. Physiol.* 251: R320-R324.

Gordon, C.J. and Fogelson, L. (1991). Comparative effects of hypoxia on behavioural thermoregulation in rats, hamsters and mice. *Am. J. Physiol.* 260: R120-R125.

Gordon, C.J.; Lee, K.A.; Chen, T.A.; Killough, P. and Ali, J.S. (1991). Dynamics of behavioural thermoregulation in the rat. *Am. J. Physiol.* 261: R705-R711.

Greishaber, M.K.; Hardewig, I.; Kreutzer, U. and Portner, H.O. (1994). Physiological and metabolic responses to hypoxia in invertebrates. *Rev. Physiol. Pharmacol.* 125: 44-139.

Grigg, G.C.; Beard, L.A. and Augee, M.L. (1989). Hibernation in a monotreme, the echidna (*Tachyglossus aculeatus*). *Comp. Biochem. Physiol. A.* 92: 609-612.

Grigg, G.; Beard, L.; Grant, T. and Augee, M. (1992). Body temperature and diurnal activity patterns in the platypus (*Ornithorhynchus anatinus*) during winter. *Aust. J. Zool.* 40: 135-142.

Guernsey, D.L. and Edelman, I.S. (1983). Regulation of thermogenesis by thyroid hormones. In: *Molecular Basis of Thyroid Hormone Action.* Eds. J.H. Oppenheimer and H.H. Samuels. Academic Press, New York. pp. 293-324.

Guiler, E.R. and Heddle, R.W.L. (1974). Body temperatures in the Tasmanian devil, *Sarcophilus harrisii* (Marsupialia: Dasyuridae). *Comp. Biochem. Physiol.* 47A: 981-989.

Gumma, M.R. and South, F.E. (1967). Temperature preference in golden hamsters. *Anim. Behav.* 15: 534-567.

Gumma, M.R. and South, F.E. (1970). Hypothermia and behavioural thermoregulation by the hamster (*Mesocricetus auratus*). *Anim. Behav.* 18: 504-511.

Gwosdow, A.R. and Besch, E.L. (1985). Effect of thermal history on the rat's response to varying environmental temperature. *J. Appl. Physiol.* 59: 413-419.

Haight, J.S.J. and Ketinge, W.R. (1973). Elevation in set-point for body temperature regulation after prolonged exercise. *J. Physiol.* 229: 77-85.

Hallman, G.M.; Ortega, C.E.; Towner, M.C. and Muchlinski, A.E. (1990). Effect of bacterial pyrogen on three lizard species. *Comp. Biochem. Physiol.* 96A(3): 383-386.

Halse, S.A. and Rose, R.W. (1988). Variation in the basal body temperature of the common brushtail possum (*Trichosurus vulpecula*) in Tasmania. *Mammalia* 52: 225-231.

Hamilton, C.L. (1959). Effect of food deprivation on thermal behaviour of the rat. *Proc. Soc. Exp. Biol. Med.* 100: 354-356.

Hammel, H. T. (1968). Regulation of internal body temperature. *Ann. Rev. Physiol.* 30: 641-710.

Hammel, H.T. (1970). Concept of the adjustable set temperature. In: *Physiological and Behavioural Temperature Regulation.* Eds. J.D. Hardy; A.P. Gagge and J.A.J. Stolwijk. C.C. Thomas, Springfield, Illinois. p. 676-683.

Hammel, H.T.; Caldwell, F.T.Jnr and Abrams, R.M. (1967). Regulation of body temperature in the blue-tongued lizard. *Science* 156: 1260-1262.

Hammel, H.T.; Jackson, D.C.; Stolwijk, J.A.J.; Hardy, T.D. and Stromme, S.B. (1963). Temperature regulation by hypothalamic proportional control with an adjustable set point. *J. Appl. Physiol.* 18: 1146-1154.

Harder, J.D.; Hsu, M.J. and Garton, D.W. (1996). Metabolic rates and body temperature of the grey short-tailed opossum (*Monodelphis domestica*) during gestation and lactation. *Physiol. Zool.* 69: 317-339.

Hart, J.S. (1971). Rodents. In: *Comparative Physiology of Thermoregulation.* Ed. G.C. Whittow. Academic Press, New York. p. 1-149.

Hayward, J.S. and Lisson, P.A. (1992). Evolution of brown fat: Its absence in marsupials and monotremes. *Can. J. Zool.* 70: 171-179.

Hellon, R.F. (1975). Monoamines, pyrogens and cations: Their actions on central control of body temperature. *Pharmacol. Rev.* 26: 289-321.

Hellon, R. (1978). Mediators and metabolic factors in thermal regulation. In: *New Trends in Thermal Physiology.* Eds. Y. Houdas and J.D. Guieu. Masson, Paris. p. 33-38.

Hensel, H. (1973). Neural processes in thermoregulation. *Physiol. Reviews* 53: 948-1009.

Hensel, H. (1981). *Thermoreception and Temperature Regulation.* Academic Press, New York.

Herrington, L.P. (1940). The heat regulation of small laboratory animals at various environmental temperatures. *Am. J. Physiol.* 129: 123.

Hicks, J.W. and Wood, S.C. (1985). Temperature regulation in lizards: Effects of hypoxia. *Am. J. Physiol.* 248: R595-R600.

Hill, J.R. (1959). The oxygen consumption of new-born and adult mammals. Its dependence on the oxygen tension in the inspired air and on the environmental temperature. *J. Physiol. Lond.* 149: 346-373.

Hinds, D.S. and MacMillen, R.E. (1984). Energy scaling in marsupials and eutherians. *Science* 225: 335-337.

Hoban, T.M.; Levine, A.H.; Shane, R.B. and Sulzman, F.M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiol. Behav.* 34: 513-518.

Hofmeyr, M.D. and Louw, G.N. (1987). Thermoregulation, pelage conductance and renal function in the desert-adapted springbok, *Antidorcas marsupialis*. *J. Arid Env.* 13: 137-151.

Holloway, J.C. and Geiser, F. (2000). Development of thermoregulation in the sugar glider *Petaurus breviceps* (Marsupialia: Petauridae). *J. Zool.* 252: 389-397.

Holloway, J.C. and Geiser, F. (2001a). Effects of helium/oxygen and temperature on aerobic metabolism in the marsupial sugar glider, *Petaurus breviceps*. *Physiol. Biochem. Zool.* 74: 219-225.

Holloway, J.C. and Geiser, F. (2001b). Seasonal changes in the thermoenergetics of the marsupial sugar glider, *Petaurus breviceps*. *J. Comp. Physiol.* 171B: 643-650.

Honma, K. and Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Jap. J. Physiol.* 28: 159-169.

Holland,K.N.; Brill, R.W.; Chang, R.K.C.; Sibert, J.R. and Fournier, D.A. (1992). Physiological and behavioural thermoregulation in bigeye tuna (*Thunnus obesus*). *Nature* 358: 410-412.

Hooper, P. and Richards, S.A. (1991). Interaction of operant behaviour and autonomic thermoregulation in the domestic fowl. *Br. Poult. Sci.* 32: 929-938.

Hope, P.J.; Daniels, C.B.; Chapman, I.; Horowitz, M.; Morley, J.E.; Trayhurn, P.; Kumaratilake, J. and Wittert, G. (1997). Identification of brown fat and mechanisms for energy balance in the marsupial, *Sminthopsis crassicaudata*. *Am. J. Physiol.* 273: R161-R167.

Horowitz, K.A.; Scott, N.R.; Hillman, P.E. and Van Tienhoven, A. (1978). Effects of feathers on instrumental thermoregulatory behaviour in chickens. *Physiol. Behav.* 21: 233-238.

Horstman, D.H. and Banderet, L.E. (1977). Hypoxia-induced metabolic and core temperature changes in the squirrel monkey. *J. Appl. Physiol.* 42: 273-278.

Hudson, J.W. and Dawson, T.J. (1975). Role of sweating from the tail in the thermal balance of the rat-kangaroo, *Potorous tridactylus*. *Aust. J. Zool.* 23: 453-461.

Hudson, R. and Distel, H. (1989). Temporal pattern of suckling in rabbit pups: a model of circadian synchrony between mother and young. In: *Development of circadian rhythmicity and photoperiodism in mammals*. Ed. S.M. Reppert. Perinatology Press, Boston. p. 83-101.

Hulbert, A.J. (1978). The thyroid hormones: A thesis concerning their action. *J. Theor. Biol.* 73: 81-100.

Hulbert, A.J. (1988). Metabolism and the development of endothermy. In: *The Developing Marsupial. Models for Biomedical Research.* Eds. C.H. Tyndale-Biscoe and P.A. Janssens. Springer-Verlag, Berlin. p.148-161.

Hulbert, A.J. (2000). Thyroid hormones and their effects: a new perspective. *Biol. Rev.* 75: 591-631.

Hulbert, A.J. and Augee, M.L. (1982). A comparative study of thyroid function in monotreme, marsupial and eutherian mammals. *Physiol. Zool.* 55: 220-228.

Hutchison, V.H. and Erskine, D.J. (1981). Thermal selection and prostaglandin E_1 fever in the salamander *Necturus maculosus. Herpetologica* 37: 195-198.

Ingram, D.L.; Walters, D.E. and Legge, K.F. (1975). Variations in behavioural thermoregulation in the young pig over 24 hour periods. *Physiol. Behav.* 14: 689-695.

Iriki, M.; Hashimoto, M. and Saigusa, T. (1987). Threshold dissociation of thermoregulatory effector responses in febrile rabbits. *Can. J. Physiol. Pharmacol.* 65: 1304-1311.

Ishii, K.; Kuwahara, M.; Tsubone, H. and Sugano, S. (1996). The telemetric monitoring of heart rate, locomotor activity, and body temperature in mice and voles (*Microtus arvalis*) during ambient temperature changes. *Lab. Anim.* 30: 7-12.

Jakubczak, L.F. (1966). Behavioural thermoregulation in young and old rats. *J. Appl. Physiol.* 21: 19-21.

Jansky, L. (1973). Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev.* 48: 85-132.

Jansky, L. (1995). Humoral thermogenesis and its role in maintaining energy balance. *Physiol. Rev.* 75: 237-259.

Janssens, P.A.; Grigg, J.A.; Dove, H. and Hulbert, A.J. (1990). Thyroid hormone during development of a marsupial, the tammar wallaby, *Macropus eugenii. J. Endocrinol.* 127: 427-436.

Jepson, M.M.; Millward, D.J.; Rothwell, N.J. and Stock, M.J. (1988). Involvement of sympathetic nervous system and brown fat in endotoxin-induced fever in rats. *Am. J. Physiol.* 255: E617-E620.

Johnson, R.W.; Propes, M.J. and Shavit, Y. (1996). Corticosterone modulates behavioural and metabolic effects of lipopolysaccharide. *Am. J. Physiol.* 39: R192-R198.

Jones, M.E.; Grigg, G.C. and Beard, L.A. (1997). Body temperatures and activity patterns of Tasmanian devils (*Sarcophilus harrisii*) and eastern quolls (*Dasyurinus viverinus*) through a subalpine winter. *Physiol. Zool.* 70: 53-60.

Jurgensen, Th. (1873). *Die Korperwarme des gesunden Menschen.* Leipzig.

Kaethner, M.M. and Good, B.F. (1975). Seasonal thyroid activity in the tammar wallaby, *Macropus eugenii.* (Desmarest). *Aust. J. Zool.* 23: 363-369.

Kamerman, P.R.; Brooksbank, R.; Pitts, N. and Laburn, H.P. (2001). Heat stress increases the rate of tolerance development to lipopolysaccharide in rats. *J. Therm. Biol.* 26: 589-594.

Kaplanski, J.; Fraifield, V. and Abramovich, I. (1994). Effect of LPS on body temperature and hypothalamic PGE_2 production in young and old rats. *Can. J. Physiol. Pharmacol.* 72 (Suppl. 1): 286.

Keil, R.; Riediel, W. and Eckhardt, S. (1996). Hormonal secretion patterns but not autonomic effector responses elicited by hypothalamic heating and cooling are altered in febrile rabbits. *J. Auton. Nerv. Syst.* 57: 193-201.

Keller, L.S.F.; Drozdowicz, C.K.; Rice, L.; Bowman, T.A. and Lang, C.M. (1988). An evaluation of three anaesthetic regimes in the gray short-tailed opossum (*Monodelphis domestica*). *Lab. Anim.* 22: 269-275.

Kittrell, E.M. And Satinoff, E. (1986). Development of the circadian rhythm of body temperature in rats. *Physiol. Behav.* 38: 99-104.

Kivivuori, L.A. (1994). Temperature selection behaviour of cold- and warm-acclimated crayfish (*Astacus astacus (L.)*). *J. Therm. Biol.* 19: 291-297.

Kleiber, M. (1971). *The Fire of Life: An introduction to animal energetics.* 2nd Edition. Huntington, New York.

Kleitman, N. (1923). Studies of the physiology of sleep. I. The effects of prolonged sleeplessness on man. *Amer. J. Physiol.* 66: 67-92.

Kleitman, N. and Satinoff, E. (1982). Thermoregulatory behaviour in rat pups from birth to weaning. *Physiol. Behav.* 29: 537-541.

Kluger, M.J. (1991). Fever: Role of pyrogens and cryogens. *Physiol. Rev.* 71: 93-127.

Kluger, M.J.; Conn, C.A.; Franklin, B.; Freter, R. and Abrams, G.B. (1990). Effect of gastrointestinal flora on body temperature of rats and mice. *Am. J. Physiol.* 258: R552-R557.

Kluger, M.J; Ringler, D.H. and Anver, M.R. (1975) Fever and survival. *Science* 188: 166-168.

Kluger, M.J.; Rudolph, K.; Soszynski, D.; Conn, C.A.; Leon, L.R.; Kozak, W.; Wallen, E.S. and Moseley, P.L. (1997). Effect of heat stress on LPS-induced fever and tumor necrosis factor. *Am. J. Physiol.* 273: R858-R863.

Korducki, M.J.; Forster, H.V.; Lowry, T.F. and Forster, M.M. (1994). Effect of hypoxia on metabolic rate in awake ponies. *J. Appl. Physiol.* 76: 2380-2385.

Kortner, G. and Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiol. Behav.* 58: 31-36.

Kortner, G. and Geiser, F. (2000). Torpor and activity patterns in free-ranging sugar gliders, *Petaurus breviceps*. *Oecologia* 123: 350-357.

Kortner, G.; Song, X. and Geiser, F. (1998). Rhythmicity of torpor on a marsupial hibernator, the mountain pygmy-possum (*Burramys parvus*) under natural and laboratory conditions. *J. Comp. Physiol. B* 168: 631-638.

Kozak, W.; Conn, C.A. and Kluger, M.J. (1994). Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. *Am. J. Physiol.* 35: R125-R135.

Kuroshima, A. (1993). Brown adipose tissue thermogenesis as physiological strategy for adaptation. *Jap. J. Physiol.* 43:117-139.

Laburn, H.P.; Mitchell, D.; Kenedi, E. and Louw, G.N. (1981). Pyrogens fail to produce fever in a cordylid lizard. *Am. J. Physiol.* 241: R198-R202.

Laudenslager, M.L. and Hammel, H.T. (1977). Environmental temperature selection by the Chukar partridge, *Alectoris chukar*. *Physiol. Behav.* 19: 543-548.

Lawson, V.J.; Curtis, A.J.; Michelangeli, V.P.; Stockigt, J.R.; Carrick, F.N. and Barlow, J.W. (1996). Concentrations of thyroid hormones and iodothyronine binding proteins in serum of the koala (*Phascolarctus cinereus*). *Aust. Vet. J.* 74: 55-58.

Leonard, C.M. (1974). Thermotaxis in golden hamster pups. *J. Comp. Physio. Psychobiol.* 86: 458-469.

Li, H. and Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *Am. J. Physiol.* 269: R208-R214.

Li, Q.; Sun, R.; Huang, C.; Wang, Z.; Liu, X.; Hou, J.; Liu, J.; Cai, L.; Li. N.; Zhang, S. and Wang, Y. (2001). Cold adaptive thermogenesis in small mammals from different geographical zones of China. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 129: 949-961.

Lipton, J.M. and Ticknor, C.B. (1979). Influence of sex and age on febrile responses to peripheral and central administration of pyrogens in the rabbit. *J. Physiol.* 295: 263-272.

Lovegrove, B.G. and Heldmaier, G. (1994). The amplitude of circadian body temperature rhythms in three rodents (*Aethomys namaquensis. Thallomys paedulous* and *Cryptomys darnarensis*) along an arboreal-subterranean gradient. *Aust. J. Zool.* 42: 65-78.

Lovegrove, B.G. (2000). The zoogeography of mammalian basal metabolic rate. *Amer. Nat.* 156: 201-219.

Macari, M.; Pela, I.R. and Silva, C.A. (1987). The development of a febrile response to pyrogen in the thyroid-deficient rabbit. *Can. J. Physiol. Pharmacol.* 65: 1325-1328.

MacMillen, R.E. and Nelson, J.E. (1969). Bioenergetics and body size in dasyurid marsupials. *Am. J. Physiol.* 217: 1246-1251.

Mailloux, A.; Benstaali, C.; Bogdan, A.; Auzeby, A. and Touitou, Y. (1999). Body temperature and locomotor activity as marker rhythms of aging in the circadian system in rodents. *Exp. Gerontology* 34: 733-740.

Maitland, S.J.; Ruwe, W.D. and Veale, W.L. (1985). Characteristics of the febrile response in old and young rats. In: *Homeostasis and Thermal Stress. Experimental and Therapeutic Advances*. Eds. K. Cooper; P. Lomax; E. Schonbaum and W.L. Veale. 6th Int. Symp. Pharmacol. Thermoregulation. p. 81-83.

Maloney, S.K. and Gray, D.A. (1998). Characteristics of the febrile response in Pekin ducks. *J. Comp. Physiol.* 168: 177-182.

Malvin, G.M. and Wood, S.C. (1991). Behavioural thermoregulation of the toad, *Bufo marinus*: Effects of air humidity. *J. Exp. Zool.* 258: 322-326.

Malvin, G.M. and Wood, S.C. (1992). Behavioural hypothermia and survival of hypoxic protozoans; *Paramecium caudatum. Science* 255: 1423-1425.

Marques, P.R.; Spencer, R.L.; Burks, T.F. and McDougal, J.N. (1984). Behavioural thermoregulation, core temperature, and motor activity: Simultaneous quantitative assessment in rats after dopamine and prostaglandin E_1. *Behav. Neurosci.* 98: 858-867.

Martin, C.J. (1903). Thermal adjustment and respiratory exchange in monotremes and marsupials. *Phil. Trans. Roy. Soc. Lond.* B195: 1-37.

Marx, J.; Hilbig, R. and Rahman, H. (1984). Endotoxin and prostaglandin E fail to induce fever in a teleost fish. *Comp. Biochem. Physiol.* 77A: 483-487.

Mawbey, R. (1989). A new trap design for the capture of sugar gliders, *Petaurus breviceps. Aust. Wildl. Res.* 16: 425-428.

McCarron, H.C.K.; Buffenstein, R.; Fanning, F.D. and Dawson, T.J. (2001). Free-ranging heart rate, body temperature and energy metabolism in eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*) in the arid regions of South East Australia. *J. Comp. Physiol. B.* 171: 401-411.

McEachron, D.L.; Lauchlan, C.L. and Midgley, D.E. (1993). Effects of thyroxine and thyroparathyroidectomy on circadian wheel running in rats. *Pharm. Biochem. Behav.* 46: 243-249.

Mendelssohn, (1895). Ueber den Thermotropismus einzeller organismen. *Pflugers Arch. Ges. Physiol.* 60: 1-27.

Milton, A.S. and Wendlandt, S. (1970). A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *J. Physiol.* 207: 76-77.

Mitchell, D.; Maloney, S.K.; Jessen, C.; Laburn, H.P.; Kamerman, P.; Mitchell, G. and Fuller, A. Adaptive heterothermy and selective brain cooling in arid-zone mammals. Unpublished Review.

Mitchell, D.; Snellen, J.W. and Atkins, A.R. (1970). Thermoregulation during fever: Change of set point or change of gain. *Pflugers Arch. Ges. Physiol.* 321: 293-302.

Mooradian, A.D.; Li, J. and Shah, G.N. (1998). Age-related changes in thyroid hormone responsive protein (THRP) expression in cerebral tissue of rats. *Brain Res.* 793: 302-304.

Morimoto, A.; Murakami, N.; Nakamori, T. and Watanabe, T. (1986). Suppression of non-shivering thermogenesis in the rat by heat-seeking behaviour during cold exposure. *J. Physiol.* 380: 541-549.

Morimoto, A.; Nakamori, T.; Watanabe, T.; Ono, T. and Murakami, N. (1988). Pattern differences in experimental fevers induced by endotoxin, endogenous pyrogen, and prostaglandins. *Am. J. Physiol.* 254: R633-R640.

Morrison, P.R. (1965). Body temperatures in some Australian mammals. IV. Dasyuridae. *Aust. J. Zool.* 13: 173-187.

Morrison, P. and Petajan, J.H. (1962). The development of temperature regulation in the opossum, *Didelphis marsupialis virginiana. Physiol. Zool.* 35: 52-65.

Mortola, J.P. and Seifert, E.L. (2000). Hypoxic depression of circadian rhythms in adult rats. *J. Appl. Physiol.* 88: 365-368.

Morton, S.R. (1978). Torpor and nest-sharing in free-living *Sminthopsis crassicaudata* (Marsupialia) and *Mus musculus* (Rodentia). *J. Mamm.* 59: 569-575.

Mory, G.; Ricquier, D.; Pesquies, P. and Hemon, P. (1981). Effects of hypothyroidism on the brown adipose tissue of adult rats: Comparison with the effects of adaptation to cold. *J. Endocr.* 91: 515-524.

Muchlinski, A.E. (1985). The energetic cost of the fever response in three species of ectothermic vertebrates. *Comp. Biochem. Physiol.* 81A: 577-579.

Muchlinski, A.E.; Baldwin, B.C. and Gramajo, R. (2000). Endotoxin elicits a febrile response in laboratory-maintained but not free-living California ground squirrels. *J. Mammal.* 81: 701-708.

Mumm, B.; Kaul, R.; Heldmaier, G. and Schmidt, I. (1990). Endogenous circadian core temperature rhythm in artificially reared week-old Wistar rats. In: *Chronobiology and Chronomedicine.* Ed. E. Morgan. Peter Lang, Frankfurt. pp. 92-101.

Myers, B.L. and Badia, P. (1995). Changes in circadian rhythms and sleep quality with aging: Mechanisms and interventions. *Neurosci. Behav. Rev.* 19: 553-571.

Myhre, K.; Cabanac, M. and Myhre, G. (1977). Fever and behavioural temperature regulation in the frog *Rana esculenta. Acta Physiol. Scand.* 101: 219-229.

Nakano, H.; Lee, S.D.; Ray, A.D.; Krasney, J.A. and Farkas, G.A. (2001). Role of nitric oxide in thermoregulation and hypoxic ventilatory response in obese zucker rats. *Am. J. Crit. Care Med.* 164: 437-442.

Naylor, A.M.; Ruwe, W.D.; Veale, W.L. and Dinarello, C.A. (1985). Influence of age on the development of fever in the rabbit. In: *Homeostasis and Thermal Stress. Experimental and Therapeutic Advances.* 6th Int. Symp. Pharmacol. Thermoregulation. Eds. K. Cooper; P. Lomax; E. Schonbaum and W.L. Veale. 6th Int. Symp. Pharmacol. Thermoregulation. p.66-69.

Nichelmann, M.; Hochel, J. And Tzschentke, B. (1999). Biological rhythms in birds – developments, insights and perspectives. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 124: 429-437.

Nicol, S.C. (1977). Thyroid function of the potoroo, *Potorous tridactylus* (Kerr). *Aust. J. Zool.* 25: 419-421.

Nicol, S.C. (1978). Non-shivering thermogenesis in the potoroo, *Potorous tridactylus* (Kerr). *Comp. Biochem. Physiol.* 59C: 33-37.

Nicol, S.C.; Andersen, N.A. and Tomasi, T.E. (2000). Seasonal variations in thyroid hormone levels in free-living exhidnas (*Tachyglossus aculeatus*). *Gen. Comp. Endocrinol.* 117: 1-7.

Nicol, S.C.; Pavlides, D. and Andersen, N.A. (1997). Nonshivering thermogenesis in marsupials: Absence of thermogenic response to β–adrenergic agonists. *Comp. Biochem. Physiol.* 117A: 399-405.

Nomoto, S. (1996). Diurnal variations in fever induced by intravenous LPS injection in pigeons. *Pflugers Arch. - Eur. J. Physiol.* 431: 987-989.

Nuesslein, B. and Schmidt, I. (1990). Development of circadian cycle of core temperature in juvenile rats. *Am. J. Physiol.* 259: R270-R276.

Nuesslein-Hildesheim, B.; Imai-Matsumura, K.; Doring, H. and Schmidt, I. (1995). Pronounced juvenile circadian core temperature rhythms exist in several strains of rats but not in rabbits. *J. Comp. Physiol. B.* 165: 13-17.

Ogilvie, D.M. and Stinson, R.H. (1966). The effect of age on temperature selection by laboratory mice (*Mus musculus*). *Can. J. Zool.* 44: 511-517.

Opazo, J.C.; Nespolo, R.F. and Bozinovic, F. (1999). Arousal from torpor in the Chilean mouse-opossum (*Thylamys elegans*): Does non-shivering thermogenesis play a role? *Comp. Biochem. Physiol.* 123A: 393-397.

Osborne, A.R. and Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *Neuroreport* 6: 2187-2192.

Ostheim, J. (1992). Coping with food-limited conditions: feeding behaviour, temperature preference and nocturnal hypothermia in pigeons. *Physiol. Behav.* 51: 353-361.

Oufara, S.; Barre, H.; Rouanet, J-L and Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *Am. J. Physiol.* 253: R39-R45.

Owen, T.L.; Spencer, R.L. and Duckles, S.P. (1991). Effect of age on cold acclimation in rats: Metabolic and behavioural responses. *Am. J. Physiol.* 260: R284-R289.

Pearson, O.P. (1960). The oxygen consumption and bioenergetics of harvest mice. *Physiol. Zool.* 33: 152-160.

Peters, D.G. and Rose, R.W. (1979). The estrous cycle and basal body temperature in the common wombat (*Vombatus ursinus*). *J. Reprod. Fert.* 57: 453-460.

Peterson, C.R. (1987). Daily variation in the body temperatures of free-ranging garter snakes. *Ecology* 68: 160-169.

Pickard, G.E.; Kahn, R. and Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiol. Behav.* 32: 763-766.

Pittman, Q.J.; Veale, W.L.; Cockeram, A.W. and Cooper, K.E. (1976). Changes in body temperature produced by prostaglandins and pyrogens in the chicken. *Am. J. Physiol.* 230: 1284-1287.

Poole. S. and Stephenson, J.D. (1977). Body temperature regulation and thermoneutrality in rats. *Q. J. Exp. Physiol.* 62: 143-149.

Prinzinger, R.; Prebmar, A. and Schleucher, E. (1991). Body temperature in birds. *Comp. Biochem. Physiol.* 99A: 499-506.

Randall, W. et al., (1987). A two-peak circadian system in body temperature and activity in the domestic cat, *Felis catus*. *J. Therm. Biol.* 12: 27-37.

Refinetti, R. (1993). Laboratory instrumentation and computing: Comparison of six methods for the determination of the period of circadian rhythms. *Physiol. Behav.* 54: 869-875.

Refinetti, R. (1995a). Body temperature and behaviour of golden hamsters (*Mesocricetus auratus*) and ground squirrels (*Spermoophilus tridecemlineatus*) in a thermal gradient. *J. Comp. Physiol.* 177: 701-705.

Refinetti, R. (1995b). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behav. Neuroscience* 109: 523-527.

Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *J. Exp. Zool.* 275: 67-70.

Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comp. Biochem. Physiol.* 119A: 295-300.

Refinetti, R. and Carlisle, H.J. (1986a). Complementary nature of heat production and heat intake during behavioural thermoregulation in the rat. *Behav. Neural Biol.* 46: 64-70.

Refinetti, R. and Carlisle, H.J. (1986b). Effects of anterior and posterior hypothalamic temperature changes on thermoregulation in the rat. *Physiol. Behav.* 36: 1099-1103.

Refinetti, R. and Horvath, S.M. (1989). Thermopreferendum of the rat: Inter- and intra-subject variabilities. *Behav. Neural Biol.* 52: 87-94.

Refinetti, R.; Ma, H. and Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Exp. Gerontol.* 25: 533-543.

Refinetti, R. and Menaker, M. (1992a). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *J. Exp. Zool.* 263: 453-457.

Refinetti, R. and Menaker, M. (1992b). The circadian rhythm of body temperature. *Physiol. Behav.* 51: 613-637.

Reichlin, S.; Martin, J.B.; Mitnick, M.; Boshans, R.L.; Grimm, Y.; Bollinger, J.; Gordon, J. and Malacara, J. (1972). The hypothalamus in pituitary-thyroid regulation. *Recent Prog. Horm. Res.* 28: 229-286.

Reynolds, W.W.; Casterlin, M.E. and Covert, J.B. (1976). Behavioural fever in teleost fish. *Nature* 259: 41-42.

Reynolds, W. and Hulbert, A.J. (1982). Cold acclimation in a small dasyurid marsupial: *Antechinuss stuartii*. In: *Carnivorous Animals*. Ed. M. Archer. Royal Zoological Society of N.S.W.

Ricciuti, F. and Fewell, J.E. (1992). Fever in young lambs: hypoxemia alters the febrile response to a small dose of bacterial pyrogen. *J. Dev. Physiol.* 17: 29-38.

Richards, S.A. (1976). Behavioural temperature regulation in the fowl. *J. Physiol.* 258: 122-123.

Richardson, C.S.; Dohm, M.R. and Garland , T. Jr (1994). Metabolism and thermoregulation in crosses between wild and random-bred laboratory house mice (*Mus domesticus*). *Physiol. Zool.* 67: 944-975.

Riedel, W. (1983). Effects of propylthiouracil, and of bacterial endotoxin (LPS), on thyroid hormones, respiratory rate, cutaneous and renal blood flow in rabbits. *Pflugers Arch.* 399: 11-17.

Riedel, W. (1990). Mechanics of fever. *J. Basic Clin. Physiol. Pharmacol.* 1: 291-322.

Rips, R.; Bellemin, J, and Boschi, G. (1989). Hypothermic effects of "pyrogens" in the mouse and interaction with lysine acetylsalicyclate. In: *Thermoregulation: Research and Clinical Applications*. Ed. P. Lomax and E. Shonbaum. Karger, Basel. p. 153-155.

Rivkees, S.A. and Reppert, S.M. (1990). Entrainment of circadian phase in developing gray short-tailed opossums: Mother vs. environment. *Am. J. Physiol.* 259: E384-E388.

Robinson, K.W. and Morrison, P.R. (1957). The reaction to hot atmospheres of various species of Australian marsupial and placental animals. *J. Cell. Comp. Physiol.* 49: 455-478.

Robinson, E.S. and Vandeberg, J.L. (1994). Blood collection and surgical procedures for the laboratory opossum (*Monodelphis domestica*). *Lab. Anim. Sci.* 44: 63-68.

Romanovsky, A.A.; Karman, E.K. and Gurin, V.N. (1992). Febrile response of the ferret to bacterial pyrogen: hypo- and hyperthermic phases. *Zh. Evol. Biokhim. Fiziol.* 28: 678-684.

Romanovsky, A.A.; Shido, O.; Sakurada, S.; Sugimotot, N. and Nagasaka, T. (1996). Endotoxin shock: Thermoregulatory mechanisms. *Am. J. Physiol.* 270: R693-R703.

Rose, R.W. (1987). Reproductive energetics of two Tasmanian rat-kangaroos (Potoroinae: Marsupialia). *Symp. Zool. Soc. Lond.* 57: 149-165.

Rose, R.W.; Kuswanti, N. and Colquhoun, E.Q. (1998). Development of endothermy in a Tasmanian marsupial, *Bettongia gaimardi* and its response to cold and noradrenaline. *J. Comp. Physiol. B.* 168: 359-363.

Rose, R.W.; Swain, R. and Bryant, S.L. (1990). Body temperature rhythm and regulation in the Tasmanian bettong (*Bettongia gaimardi*) (Marsupialia: Potoroidae). *Comp. Biochem. Physiol.* 97A: 573-576.

Rose, R.W.; West, A.K.; Ye, J.M.; McCormick, G.H. and Colquhoun, E.Q. (1999). Nonshivering thermogenesis in a marsupial (the Tasmanian bettong, *Bettongia gaimardi*) is not attributable to brown adipose tissue. *Physiol. Biochem. Zool.* 72: 699-704.

Rosendorff, C. and Mooney, J.J. (1971). Central nervous system sites of action of a purified leucocyte pyrogen. *Am. J. Physiol.* 220: 597-603.

Rosenthal, M.; Roth, J.; Storr, B. and Zeisberger, E. (1996). Fever response in lean (Fa/-) and obese (fa/fa) Zucker rats and its lack to repeated injections of LPS. *Physiol. Behav.* 59: 787-793.

Roth, J.; McClellan, J.L.; Kluger, M.J. and Zeisberger, E. (1994). Attenuation of fever and release of cytokines after repeated injections of lipopolysaccharide in guinea-pigs. *J. Physiol.* 477: 177-185.

Rounsevell, D.E.; Taylor, R.J. and Hocking, G.J. (1991). Distribution records of native terrestrial mammals in Tasmania. *Aust. Wildl. Res.* 18: 699-717.

Rozin, P. and Mayer, J. (1961). Thermal reinforcement and thermoregulatory behaviour in the goldfish, *Carcissius auratus*. *Science* 134: 942-943.

Ruben, J. (1996). Evolution of endothermy in mammals, birds and their ancestors. In: *Animals and Temperature: Phenotypic and Evolutionary Adaptation.* Eds. I.A. Johnston and A.F. Bennett. Cambridge University Press, Cambridge. p. 347-377.

Sachdeva, U. and Jennings, D.B. (1994). Effects of hypercapnia on metabolism, temperature, and ventilation during heat and fever. *J. Appl. Physiol.* 76: 1285-1292.

Sacher, G.A. and Duffy, P.H. (1978). Age changes in rhythms of energy metabolism, activity and body temperature in *Mus musculus* and *Peromyscus*. In: *Aging and Biological Rhythms* Eds. H.V. Samis and A. Capobianio. Plenum Press, New York. p. 105-124.

Saiki, C. and Mortola, J. (1996). Effect of CO_2 on the metabolic and ventilatory responses to ambient temperature in conscious adult and newborn rats. *J. Physiol.* 491: 261-269.

Satinoff, E. and Henderson, R. (1977). Thermoregulatory behaviour. In: *Handbook of Operant Behaviour*. Eds. W.K. Honig and E.R. Staddon. Prentice-Hall, Englewood Cliffs, NJ. p. 153-173.

Satinoff, E.; Peloso, E. and Plata-Salamn, C.R. (1999). Prostaglandin E_2-induced fever in young and old Long-Evans rats. *Physiol. Behav.* 67: 149-152.

Satinoff, E. and Rutstein, J. (1970). Behavioural thermoregulation in rats with anterior hypothalamic lesions. *J. Comp. Physiol. Psychobiol.* 71: 77-82.

Scales, W.E. and Kluger, M.J. (1987). Effect of antipyretic drugs on circadian rhythm in body temperature of rats. *Am. J. Physiol.* 253: R306-R313.

Scammell, T.E.; Elmquist, J.K. and Saper, C.B. (1996). Inhibition of nitric oxide synthase produces hypothermia and depresses lipopolysaccharide fever. *Am. J. Physiol.* 271: R333-R338.

Scarpace, P.J.; Matheny, M.; Bender, B.S. and Borst, S.E. (1992). Impaired febrile response with age: Role of thermogenesis in brown adipose tissue. *Proc. Soc. Exp. Biol. Med.* 200: 353-358.

Schmidt, I. (1978). Interactions of behavioural and autonomic thermoregulation in heat stressed pigeons. *Pfluegers Arch.* 374: 47-55.

Schmidt, I. (1984). Interactions of behavioural and autonomic thermoregulation. In: *Thermal Physiology.* Ed. J.R.S. Hales. Raven, New York. p. 309-318.

Schmidt, I.; Barone, A. and Carlisle, H.J. (1986). Diurnal cycle of core temperaure in huddling, week old rat pups. *Physiol. Behav.* 37: 105-109.

Schmidt, I.; Kaul, R. and Heldmaier, G. (1987). Thermoregulation and diurnal rhythms in 1-week-old rat pups. *Can. J. Physiol. Pharmacol.* 65: 1355-1364.

Schmidt, I. and Rautenberg, W. (1975). Instrumental thermoregulatory behaviour in pigeons. *J. Comp. Physiol.* 101: 225-235.

Schmidt, I. and Simon, E. (1979). Interaction of behavioural and autonomic thermoregulation in cold exposed pigeons. *J. Comp. Physiol.* 133: 151-157.

Scholander, P.F.; Hock, R.; Walters, V.; Johnson, F. and Irving, L. (1950). Heat regulation in some arctic and tropical mammals and birds. *Biol. Bull. Mar. Biol. Lab.* 99: 237-258.

Schurmann, H.; Steffensen, J.F. and Lomholt, J.P. (1991). The influence of hypoxia on the preferred temperature of rainbow trout, *Oncorhynchus mykiss. J. Exp. Biol.* 157: 75-86.

Schwartz, H.L. (1983). Effect of thyroid hormone on growth and development. In: *Molecular Basis of Thyroid Hormone Action.* Eds. J.H. Oppenheimer and H.H. Samuels. Academic Press, New York. pp. 413-444.

Sehic, E.; Li, S.; Ungar, A.L. and Blatteis, C.M. (1998). Complement reduction impairs the febrile response of guinea pigs to endotoxin. *Am. J. Physiol.* 274: R1594-R1603.

Sellers, E.A. and You, S.S. (1950). Role of the thyroid in metabolic response to a cold environment. *Am. J. Physiol.* 163: 81-91.

Setchell, P.J. (1974). The development of thermoregulation and thyroid function in the marsupial, *Macropus eugenii* (Desmarest). *Comp. Biochem. Physiol.* 47A: 1115-1121.

Severinsen, T. and Oritsland, N.A. (1991). Endotoxin induced prolonged fever in rats. *J. Therm. Biol.* 16(3): 167-171.

Shemi, D. and Kaplanski, J. (1995). Effect of different ambient temperatures and lipopolysaccharide administration on the circadian rhythm of rectal temperature and hypothalamic PGE_2 production in aged male rats. *Mech. Ageing Dev.* 85: 65-72.

Shido, O.; Sakurada, S.; Hashimoto, T.; Fujikake, K. and Nagasaka, T. (1993). Increase in plasma thyroid hormone levels during the previous heat exposure time in heat-acclimated rats. *Can. J. Physiol. Pharmacol.* 71: 904-909.

Shido, O.; Sakurada, S.; Sugimoto, N.; Hiratsuka, Y. and Takuwa, Y. (2001). Ambient temperatures preferred by humans acclimated to heat given at a fixed daily time. *Physiol. Behav.* 72: 387-392.

Shield, J. (1966). Oxygen consumption during pouch development of the macropod marsupial *Setonix brachyurus. J. Physiol.* 187: 257-270.

Shoemaker, J.A. and Refinetti, R. (1996). Day-night difference in the preferred ambient temperature of human subjects. *Physiol. Behav.* 59: 1001-1003.

Shupnik, M.A.; Ridgway, E.C. and Chin, W.W. (1989). Molecular biology of thyrotropin. *Endocr. Rev.* 10: 459-475.

Sievert, L.M. and Hutchison, V.H. (1988). Light versus heat: Thermoregulatory behaviour in a nocturnal lizard (*Gecko gecko*). *Herpetologica* 44: 266-273.

Sievert, L.M. and Paulissen, M.A. (1996). Temperature selection and thermoregulatory precision of bisexual and parthenogenetic *Cnemidophorus* lizards from Southern Texas, U.S.A. *J. Therm. Biol.* 21: 15-20.

Silva, J.E. (1995). Thyroid control of thermogenesis and energy balance. *Thyroid* 5: 481-492.

Slotkin, T.A. and Sleptis, R.J. (1984). Obligatory role of thyroid hormones in the development of peripheral sympathetic and central nervous system catecholaminergic neurone: Effects of propylthiouracil-induced hypothyroidism on transmitter levels, turnover and release. *J. Pharmacol. Exp. Ther.* 230: 53-61.

Smith, B.K. and Dawson, T.J. (1984a). Changes in the thermal balance of a marsupial (*Dasyuroides byrnei*) during cold and warm acclimation. *J. Therm. Biol.* 9: 199-204.

Smith, B.K. and Dawson, T.J. (1984b). Effect of cold and warm acclimation on the thermal balance of a marsupial (*Dasyuroides byrnei*). In: *Thermal Physiology* Ed. J.R.S. Hales. Raven, New York. p.475-478.

Smith, B.K. and Dawson, T.J. (1985). Use of helium-oxygen to examine the effect of cold acclimation on the summit metabolism of a marsupial, *Dasyuroides byrnei. Comp. Biochem. Physiol.* 81A: 445-449.

Smith, D.A. and Fisher, K.C. (1956), The distribution, orientation, and activities of the varying lemming in a gradient of temperature. *Can. J. Zool.* 34: 243-261.

Song, X.; Kortner, G. and Geiser, F. (1998). Temperature selection and use of torpor by the marsupial *Sminthopsis macroura. Physiol. Behav.* 64: 675-682.

Soszynski, D. (2001). The inhibition of nitric oxide synthase suppresses LPS - a psychological-stress-induced fever in rats. *Physiol. Behav.* 72: 65-72.

Soszynski, D.; Kozak, W. and Szewczenko, M. (1991). Course of fever response to repeated administration of sublethal doses of lipopolysaccharides, polyinosinic:polycytidylic acid and muramyl dipeptide to rabbits. *Experentia* 47: 43-47.

Steiner, A.A. and Branco, L.G.S. (2001). Nitric oxide in the regulation of body temperature and fever. *J. Therm. Biol.* 26: 325-330.

Stephenson, L.A.; Wenger, C.B.; O'Donovan, B.H. and Nadel, E.R. (1984). Circadian rhythm in sweating and cutaneous blood flow. *Am. J. Physiol.* 246: R321-R324.

Stinson, R.H. and Fisher, K.C. (1953). Temperature selection in deer mice. *Can. J. Zool.* 31: 404-416.

Stitt, J.T. and Shimada, S.G. (1987). A comparison of the febrile responses of the Brattleboro and Sprague-Dawley strains of rats to endotoxin and endogenous pyrogen. *Can. J. Physiol. Pharmacol.* 65: 1377-1381.

Stitt, J.T.; Shimada, S.G. and Bernheim, H.A. (1985). Comparison of febrile responsiveness of rats and rabbits to endogenous pyrogen. *J. Appl. Physiol.* 59: 1721-1725.

Strahan, R. (1987). *What Mammal is that?* Angus and Robertson, North Ryde, NSW.

Streilein, K.E. (1982). Behaviour, ecology, and distribution of South American marsupials. In: *Mammalian Biology in South America.* Eds. M.A. Mores and H.H. Genoways. Pymatuning Laboratory of Ecology; Special publication. Linesvale, PA. p. 231-249.

Strijbos, P.J.; Horan, M.A.; Carey, F. and Rothwell, N.J. (1993). Impaired febrile responses of aging mice are mediated by endogenous lipocortin-1 (annexin-1). *Am. J. Physiol.* 265: E289-E297.

Sugimoto, N.; Shido, O.; Sakurada, S. and Nagasaka, T. (1996). Day-night variations of behavioural and autonomic thermoregulatory responses to lipopolysaccharide in rats. *Jpn. J. Physiol.* 46: 451-456.

Sugimoto, N.; Sakurada, S. and Shido, O. (1999). Selected ambient temperatures of rats acclimated to heat given on various schedules. *Pflugers Arch.* 438: 766-770.

Swiergiel, A.H. (1987). Decrease in body temperature and locomotor activity as an adaptational response in piglets exposed to cold on restricted feeding. *Physiol. Zool.* 40: 117-125.

Szekely, M. (1984). Changed set-point and changed sensitivity in fever: Either or both? In: *Thermal Physiology* Ed. J.R.S. Hales. Raven, New York. p. 525-530.

Szelenyi, Z. and Donhoffer, S. (1968). The thermogenic function of brown adipose tissue and the response of body temperature to hypoxia and hypercapnia in the cold- and warm-adapted rat. *Acta Physiol. Acad. Sci. Hungaricae* 33: 31-39.

Szymusiak, R.; DeMory, A.; Kittrell, E.M.W. and Satinoff, E. (1985). Diurnal changes in thermoregulatory behaviour in rats with medial preoptic lesions. *Am. J. Physiol.* 249: R219-R227.

Terai, Y.; Asayama, M.; Ogawa, T.; Sugenoya, J. and Miyagawa, T. (1985). Circadian variation of preferred environmental temperature and body temperature. *J. Therm. Biol.* 10: 151-156.

Thompson, S.D. and Nicoll, M.E. (1986). Basal metabolic rates and energetics of reproduction in therian mammals. *Nature* 321: 690-693.

Tocco-Bradley, R.; Kluger, M.J. and Kauffman, C.A. (1985). Effect of age on fever and acute-phase response of rats to endotoxin and *Salmonella typhimurium*. *Infect. Immun.* 47: 106-111.

Toien, O. and Mercer, J.B. (1998). Thermogenic responses to body cooling during fever induced by *Staphylococcus aureus* cell walls in rabbits. *J. Comp. Physiol. B* 168: 73-80.

Tomasi, T.E. (1991). Mini Review: Utilisation rates of thyroid hormones in mammals. *Comp. Biochem. Physiol.* 100A: 503-516.

Tosini, G. and Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *J. Biol. Rhythms* 10: 248-255.

Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioural thermoregulation in the green iguana. *J. Comp. Physiol. A.* 179: 135-142.

Trayhurn, P. (1993). Brown adipose tissue: From thermal physiology to bioenergetics. *J. Biosci.* 18(2): 161-173.

Treagust, D.F.; Randall, W. and Folk, Jr. G.E. (1980). A Fourier regression analysis of body temperature of the American opossum, *Didelphis virginiana*. *J. Interdiscipl. Cycle Res.* 11: 132-144.

Triandafillou, J.; Gwilliam, C. and Himms-Hagen, J. (1982). Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria. *Can. J. Biochem.* 60: 530-537.

Underwood, H.; Steele, C.T. and Zivkovic, B, (1999). Effects of fasting on the circadian body temperature rhythm of Japanese quail. *Physiol. Behav.* 66: 137-143.

Vargas, M.; Jimenez, D.; Leon-velarde, F.; Osorio, J. and Mortola, J.P. (2001). Circadian patterns in men acclimatised to intermittent hypoxia. *Respir. Physiol.* 126: 233-243.

Vaughn, L.K.; Veale, W.L. and Cooper, K.E. (1980). Response of rabbits to pyrogen in a helox environment. *J. Therm. Biol.* 5: 203-206.

Vessotskie, J.M.; McGonigle, P.; Molthen, R.C. and McEachron, D.L. (1993). Thyroid and thyroxine effects on adrenoreceptors in relation to circadian activity. *Pharm. Biochem. Behav.* 46: 251-257.

Wallis, R.L. (1979). Responses to low temperature in small marsupial mammals. *J. Therm. Biol.* 4: 105-11.

Wakabayashi, G.; Cannon, J.G.; Gelfand, J.A.; Clark, B.D.; Aiura, K.; Burke, J.F.; Wolff, S.M. and Dinarello, C.A. (1994). Altered interleukin-1 and tumor necrosis factor production and secretion during pyrogenic tolerance to LPS in rabbits. *Amer. J. Physiol.* 267: R329-R336.

Webb, P. (1995). The physiology of heat regulation. *Am. J. Physiol.* 268: R838-R850.

Weiss, B. (1957). Thermal behaviour of the subnourished and pantothenic acid-deprived rat. *J. Comp. Physiol. Psychol.* 59: 481-485.

Weiss, B. and Laties, V.G. (1961). Behavioural thermoregulation. *Science* 133: 1338-1344.

Wells, R.T. (1978). Thermoregulation and activity rhythms in the hairy-nosed wombat, *Lasiorhinus latifrons* (Owen), (Vombatidae). *Aust. J. Zool.* 26: 639-651.

Whitaker, E.M.; Hussain, S.H.; Hervey, G.R.; Tobin, G. and Rayfield, K.M. (1990). Is increased metabolism in rats in the cold mediated by the thyroid? *J. Physiol. Lond.* 431: 543-556.

Wilkinson, M.F. and Kasting, N.M. (1990). Centrally acting vasopressin contributes to endotoxin tolerance. *Amer. J. Physiol.* 258: R443-R449.

Wilkinson, M.F. and Pittman, Q.J. (1994). Alteration of the physiological responses to indomethacin by endotoxin tolerance in the rat: A role for central vasopressin. *J. Physiol. (Lond)* 479: 441-449.

Withers, K.W. and Hulbert, A.J. (1988). Cold acclimation in the marsupial *Antechinus stuartii*: Thyroid function and metabolic rate. *Aust. J. Zool.* 36: 421-427.

Wood, S.C. (1991). Interactions between hypoxia and hypothermia. *Ann. Rev. Physiol.* 53: 71-85.

Wood, S.C. and Gonzales, R. (1996). Hypothermia in hypoxic animals: Mechanisms, mediators, and functional significance. *Comp. Biochem. Physiol.* 113B: 37-43.

Wood, S.C.; Hicks, J.W. and Duprè, R.K. (1987). Hypoxic reptiles: Blood gases, body temperature and control of breathing. *Amer. Zool.* 27: 21-29.

Wood, S.C. and Malvin, G.M. (1991). Physiological significance of behavioural hypothermia in hypoxic toads (*Bufo marinus*). *J. Exp. Biol.* 159: 203-215.

Wood, S.C. and Malvin, G.M. (1992) Behavioural hypothermia: An adaptive stress response. In: *Physiological Adaptations in Vertebrates: Respiration, Circulation, and Metabolism.* Eds. S.C. Wood; R.E. Weber; A.R. Hargens and R.W. Millard. Marcel Dekker, New York. pp.295-312.

Wood, S.C. and Stabenau, E.K. (1998). Effect of gender on thermoregulation and survival of hypoxic rats. *Clin. Exp. Pharmacol. Physiol.* 25: 155-158.

Ye, J.M.; Edwards, S.J.; Rose, R.W.; Rattigan, S.; Clark, M.G. and Colquhoun, E.Q. (1995). Vasoconstrictors alter oxygen, lactate and glycerol metabolism in the perfused hindlimb of a rat kangaroo. *Am. J. Physiol.* 268: R1217-R1223.

Yoda, T.; Crawshaw, L.I.; Yoshida, K.; Su, L.; Hosono, T.; Shido, O.; Sakurada, S.; Fukuda, Y. and Kanosue, K. (2000). Effects of food deprivation on daily changes in body temperature and behavioural thermoregulation in rats. *Am. J. Physiol. Regul. Int. Comp. Physiol.* 278: R134-R139.

Yousef, M.K. and Johnson, H.D. (1975). Thyroid activity in desert rodents: a mechanism for lowered metabolic rate. *Am. J. Physiol.* 229: 427-431.